THE CASSAVA HANDBOOK

A Reference Manual based on the Asian Regional Cassava Training Course held in Thailand

The Nippon Foundation

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Centro Internacional de Agricultura Tropical
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A Reference Manual based on the Asian Regional Cassava Training Course, held in Thailand

Editor: R.H. Howeler

Organized by the Centro Internacional de Agricultura Tropical (CIAT), the Department of Agriculture DOA) and the Thai Tapioca Development Institute (TTDI) of Thailand

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Cover Photos:
Top: R.H. Howeler: Iron deficiency in cassava planted on old termite hills in Huay Bong, Thailand

Bottom: R.H. Howeler: Cassava intercropped with peanut and with hedgerows of vetiver grass and other species in an FPR erosion control trial in Dong Rang, Hoa Binh, North Vietnam

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I. Cassava Situation in Asia, Cassava Physiology, Breeding, Biotechnology, Pests and Diseases, Conducting Cassava Experiments, Mineral Nutrition, Soil Fertility Maintenance, Erosion Control, Agronomy, Farmer Participatory Research, Use of Cassava Roots and Leaves for Animal Feeding, Small-scale Cassava Processing into Ethanol
II. Howeler, R.H.
III. Centro Internacional de Agricultura Tropical

PREFACE

This Handbook is the result of a combined effort by several current and previous cassava researchers at CIAT to review and summarize the most important results of cassava research during the past 40 years. Most of the chapters are based on the various presentations during the Regional Cassava Training Course, held in Thailand from October 6 to 17, 2008. This course was organized upon the realization that many people that were actively involved with cassava research in the 1970s and 80s, both at CIAT and in national programs, have now retired or will soon do so, and that a whole new generation of cassava researchers are currently being hired to take their place.

As cassava is now becoming a very important, and mostly industrial, crop in Asia, there are many new opportunities, but also a host of new problems and challenges. These include the appearance in Asia of new cassava diseases and pests; the decreasing availability and increasing cost of rural labor, resulting in the need for partial or complete mechanization of cassava production; the rapidly increasing demand for cassava roots for production of food, feed and fuel, and the unavailability in many countries of new land for any expansion of cassava area, thus requiring a rapid increase in cassava yields to increase supplies. This requires a renewed focus on cassava research for the development of new higher-yielding varieties and more sustainable production practices.

While many cassava researchers in national programs in Asia received individual or group training at CIAT-Colombia during the 1970s and 80s, this training was greatly reduced during the following two decades due to funding limitations. Thus, the objective of the Regional Cassava Training Course in 2008 was to provide a new training opportunity for young scientists in Asian countries, and to hand over the knowledge and experience of the older cassava researchers, mostly from CIAT, to a new generation that will have to face the new challenges. Thus, the course was taught mostly by current or already retired CIAT cassava researchers, while the 60-plus participants of the course included cassava researchers from Cambodia, China, East Timor, India, Indonesia, Lao PDR, Malaysia, Myanmar, the Philippines, Thailand and Vietnam. The training course not only provided knowledge –from physiology and biotechnology to animal feeding and production of fuel-ethanol – but also an opportunity for people from different institutions and countries to get to know each other, which will greatly facilitate future collaboration.

Course participants returned home with a CD containing the PowerPoint presentations of the course. However, it was felt that a more comprehensive review of all the topics covered was warranted, as this would provide more in-depth knowledge for those working in the various specialized fields. Most of this information is available in many refereed journals, in workshop proceedings and old CIAT annual reports, but many of these are now out of print or otherwise difficult to obtain. Thus, the Cassava Handbook is a first attempt to review and summarize the nearly 40 years of cassava research, and to bring together this information in one publication that can serve as a reference manual for those charged with current and future research on cassava in Asia, as well as in other parts of the world. I hope this publication is useful and I wish you well in this endeavor.

Reinhardt Howeler
November, 2011

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CHAPTER 1

RECENT TRENDS IN PRODUCTION AND UTILIZATION OF CASSAVA IN ASIA [1]

Reinhardt H. Howeler[2]

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) has its origin in Latin America where it has been grown by the indigenous Indian population for at least 4000 years. After the discovery of the Americas, European traders took the crop to Africa as a potentially useful food crop; later it was also taken to Asia to be grown as a food security crop and for the extraction of starch. Thus, in the 19th century cassava became an important food crop in southern India, as well as on Java island of Indonesia and in the southern Philippines, while in Malaysia and parts of Indonesia it was also used for extraction of starch. After the Second World War it became an important industrial crop in Thailand, mainly to produce starch for local consumption, and dried chips and later pellets for the rapidly growing European animal feed market. In Indonesia the crop remains first and foremost a food crop, used in a great variety of dishes, but in southern Sumatra it is now mainly grown for starch extraction.

PRESENT SITUATION

1. Cassava Production Trends

Table 1 indicates that in 2008/09 about 51% of cassava in the world was produced in Africa, 35% in Asia, and only 14% in Latin America and the Caribbean (**Figures 1 and 2)**

Cassava production in Asia increased at a high rate of 3% annually during the lately 70s and early 80s, slowed down during the 90s, and has been growing quite rapidly again at 5.6% per year during the past ten years, and at a very high rate of 9.1% during the past 5 years. This was the result of a modest increase in area, but was mainly driven by a remarkable increase in yields, averaging 3.7% per year during the past ten years; the latter compares with annual yield increases of only 1.3% in Africa and 0.4% in Latin America during the same period **(Figure 3)**.

Figure 4 shows the production and yield in the main cassava producing countries in Asia from 1961 to 2008. In some countries, cassava production kept pace with increases in population, while in others it decreased as a result of rapid urbanization and a more secure supply of the preferred food, rice.

A marked exception is Thailand, where cassava production increased rapidly in the 1970s and 80s in response to a rapidly growing demand for animal feed in Europe, as well as a favorable tariff structure. But when the Common Agricultural Policy (CAP) in the EU changed in the late 80s, cassava became less competitive with locally produced barley, and exports of cassava pellets declined rapidly, from a peak of 9.1 million tons in 1989 to less than 400,000 tons in 2009 (**Figure 5**). This near-collapse of the export market in Europe

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[1] This chapter is an updated and shortened version of Howeler, 2010a.

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was partially offset by accelerated growth in the production of starch and starch derivatives, as well as by increasing demand for cassava chips in China.

Table 1. Cassava production, area, and yield in the world, the continents and in various countries in Asia in 2008/09.

	Production ('000 tons)	Area ('000 ha)	Yield (t/ha)
World	**233,796**	**18,917**	**12.35**
-Africa	118,862 (51%)	12,260	9.69
-Americas	33,145 (14%)	2,588	12.81
-Asia	81,620 (35%)	4,053	20.14
-Cambodia	3,497	157	22.27
-China	4,506	269	16.27
-India	9,623	280	34.37
-Indonesia	22,039	1,176	18.75
-Laos	153	10	14.71
-Malaysia	440	42	10.47
-Myanmar	355	27	13.40
-Philippines	2,044	216	9.47
-Sri Lanka	278	24	11.64
-Thailand	30,088	1,327	22.68
-Timor-Leste	37	9	4.14
-Vietnam	8,557	509	16.82

Source: *FAOSTAT, Oct 2011.*

Meanwhile, in Vietnam, cassava production was in decline during the 1980s and 1990s as the economy improved and production of rice increased. But during the past ten years, cassava production suddenly increased from about 2 million tons in 2000 to over 8.5 million tons in 2009, in order to meet buoyant internal demand for starch, and for export of chips and starch. This ability to increase production was a result of a substantial increase in planted area, from 237,600 ha in 2000 to 508,800 ha in 2009, as well as a remarkable increase in yield, from 8.36 t/ha in 2000 to 16.82 t/ha in 2009 (**Figure 4)**.

In both Thailand and Vietnam, the yield increases achieved during the past ten years are mainly due to a concerted effort to distribute widely the new high-yielding and high-starch varieties, as well as to the adoption of improved cultural practices, such as more balanced fertilizer use and soil conservation measures. In Thailand, new varieties are now planted in nearly 100% of the area, while 80-90% of farmers apply chemical fertilizers; in Vietnam the new varieties are now planted in about 60% of the cassava area while about 80% of farmers apply chemical and/or organic manures. These two factors combined nearly doubled yields in Vietnam over the past ten years. But, the most remarkable increases have occurred in Cambodia, where cassava production increased 30 times between 2001/02 and 2010/11, from 142,262 to 4,248,942 tons, due to a doubling of yields and a 15 times increase in the cassava area. Cassava has become the second most important crop in Cambodia, after rice.

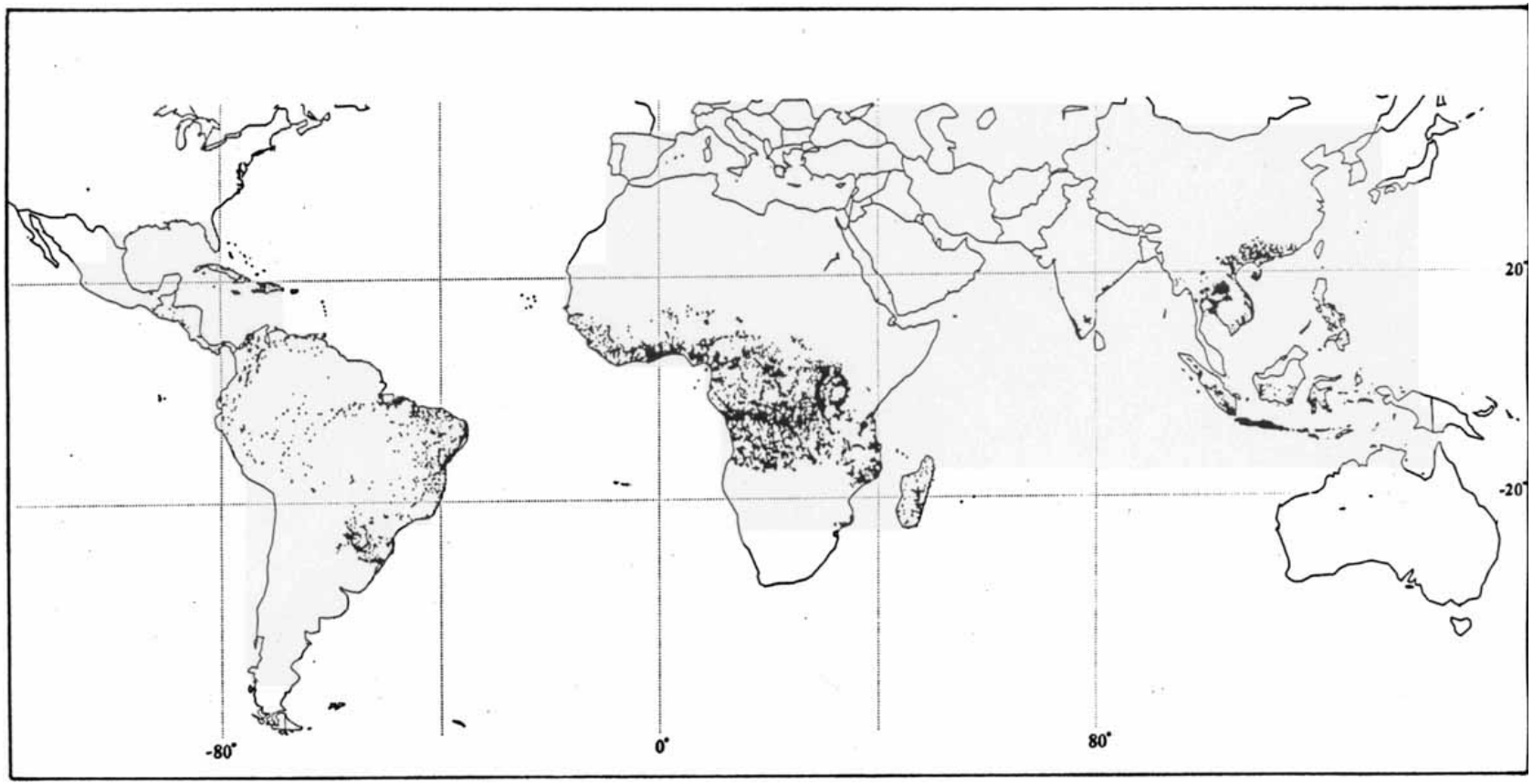

Figure 1. Distribution of cassava in the world. Each dot represents 1,000 ha.
Source: *Henry and Gottret, 1996.*

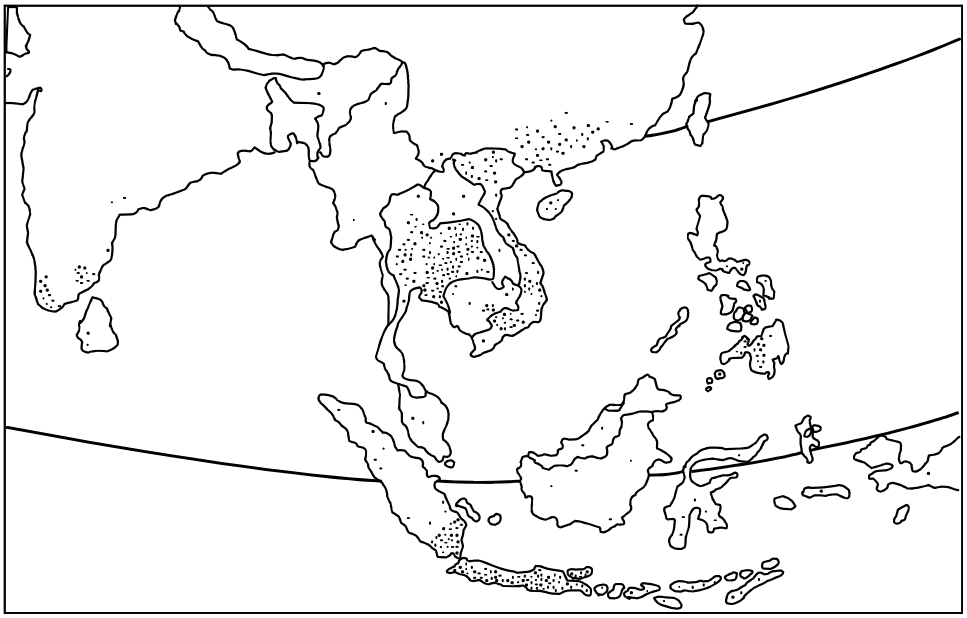

Figure 2. Cassava production zones in Asia in 2007. Each dot represents 10,000 ha of cassava.

2. Production Systems

Cassava is known to be a very drought-tolerant and water-efficient crop, while the crop is also exceptionally tolerant of high soil acidity and low levels of available phosphorus (P). Thus, cassava can compete with other, more valuable, crops such as maize, soybean and vegetables mainly in areas of acid and low-fertility soils, and those with low or unpredictable rainfall, such as the northeast of Thailand, the central coast of Vietnam and in east Java or southern Sumatra in Indonesia.

Farm size in Asia tends to be very small, with areas under cassava ranging from 0.2-0.8 ha/family in China, Vietnam, Kerala state of India and Java island in Indonesia, to 2-3 ha/family in Thailand **(Table 2)**.

The crop is often grown in association with maize, upland rice and grain legumes in Indonesia, with peanut or black beans (cowpea) in North Vietnam, with peanut or watermelon in Guangxi province and with young rubber trees in Hainan province of China, and under coconut trees in the Philippines and Kerala state of India. It is primarily grown in monoculture in Thailand, Cambodia, Malaysia and South Vietnam.

The land is usually prepared by hand (hoe) in Kerala state of India, in Java island of Indonesia, in Lao PDR and in Myanmar; by cattle or buffalo in north Vietnam, China, Tamil Nadu state of India and in Lampung province of Indonesia; and by tractor in Thailand, south Vietnam, Malaysia and in Cambodia.

In India, Indonesia and Thailand cassava stakes are mostly planted vertically, while in China, Vietnam and Cambodia they are mostly planted horizontally or inclined.

Fertilizers or organic manures are commonly used on cassava, but not necessarily in adequate amounts or in the right proportions of N, P and K. Usually, responses to organic manures can be greatly enhanced by additional application of chemical fertilizers high in N and K.

Cassava is generally weeded by hand (hoe) 2-3 times during the first 3-4 months, but herbicides are now commonly used in Thailand, China and Malaysia (**Table 2**).

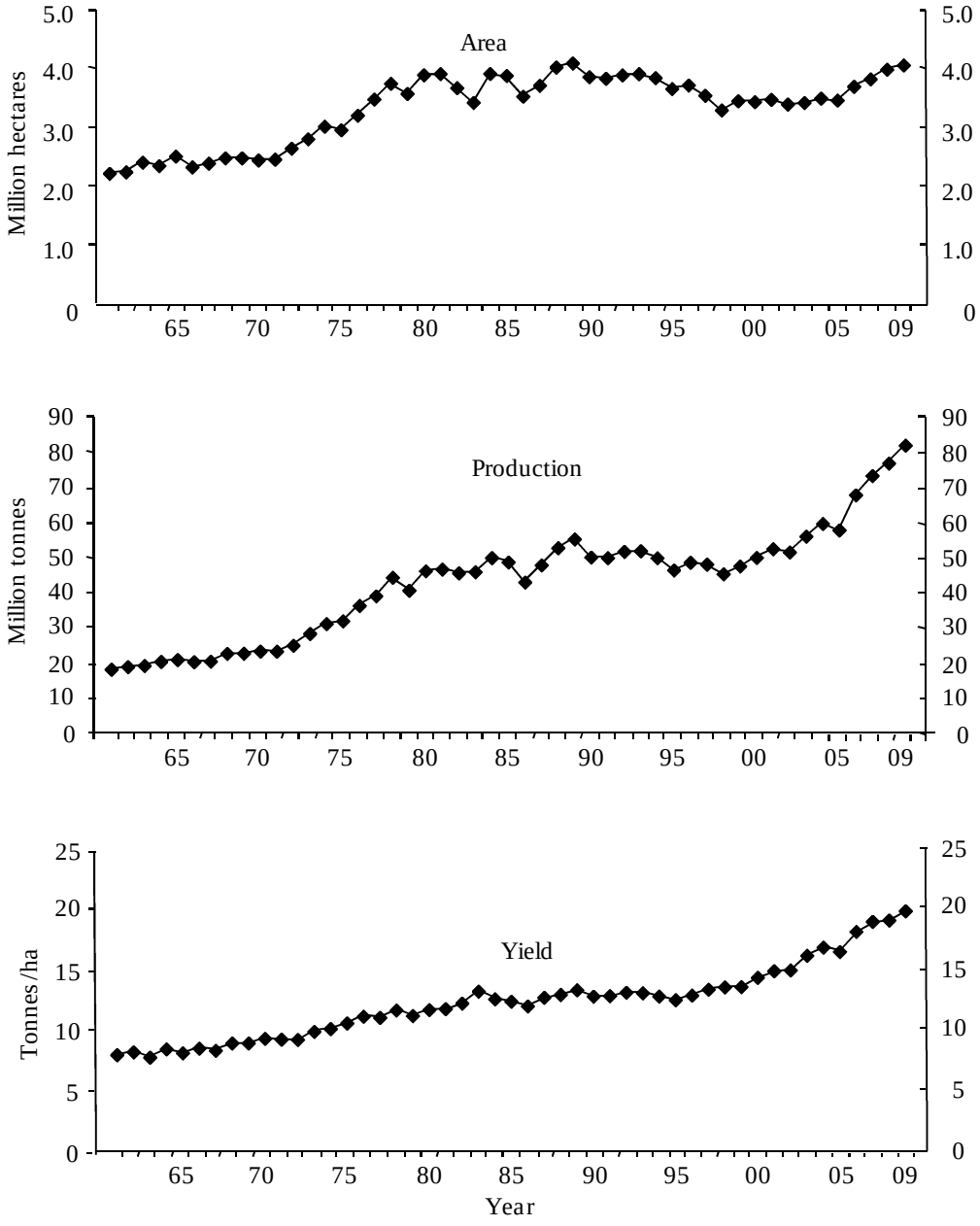

Figure 3. Total harvested area, production and yield of cassava in 12 cassava growing countries in Asia, 1961-2009.
***Source**: FAOSTAT, Oct 2011.*

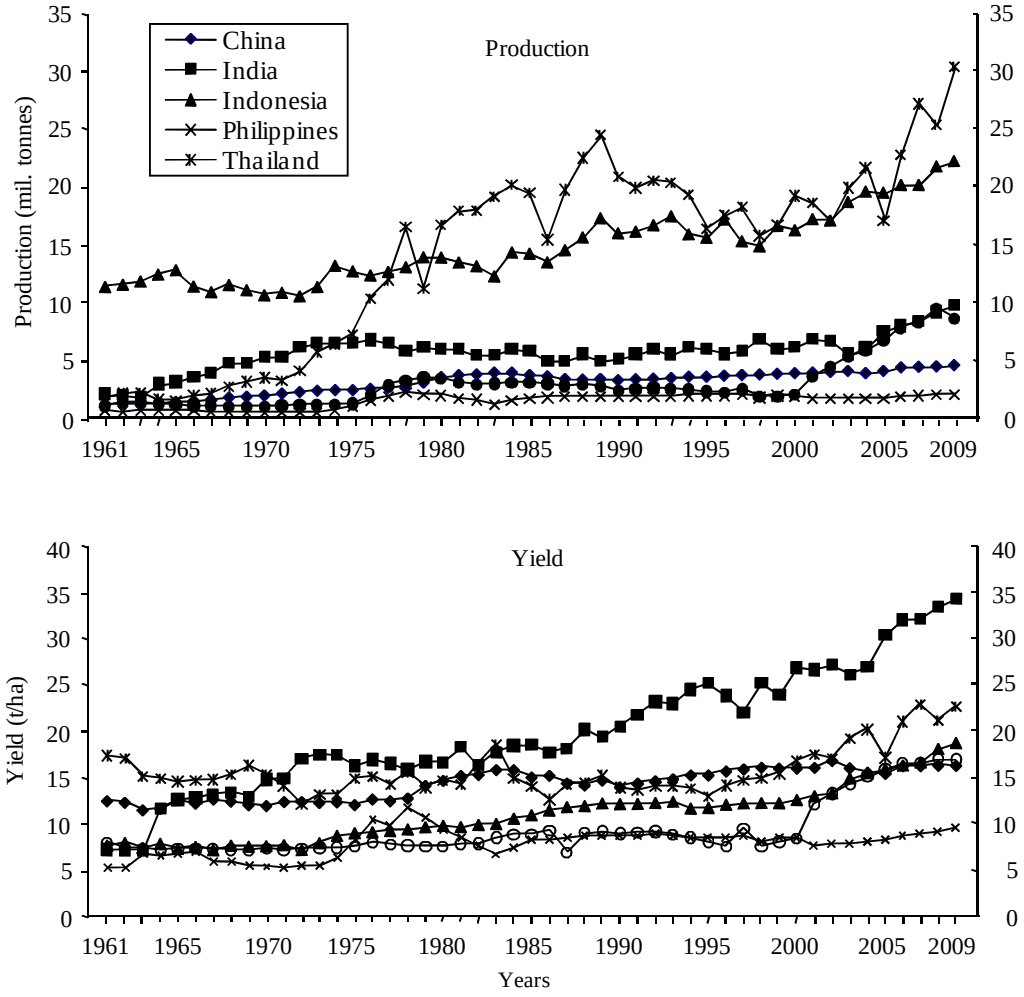

Figure 4. Cassava production and yield trends in Asia's principal cassava producing countries, 1961-2009.
***Source**: FAOSTAT, Oct 2011.*

Production costs vary significantly across the region. Production costs for cassava farmers in China, India, Indonesia and Lao PDR are higher than in Thailand, Philippines and Cambodia, which in turn are higher than in Vietnam. When calculated per ton of fresh roots, production costs in Thailand are slightly higher than in Vietnam, but much lower than in the Philippines, Lao PDR, Indonesia and China. It is clear that cassava products from Vietnam and Thailand remain competitive in the world market as farmers have increased their yields through the use of improved varieties and better production practices (Howeler, 2001; 2005; 2010). Cassava yields in India are by far the highest in the world, but due to high production costs, the cost per ton of cassava produced is still fairly high, making it difficult for India to compete on the world market (**Table 2**).

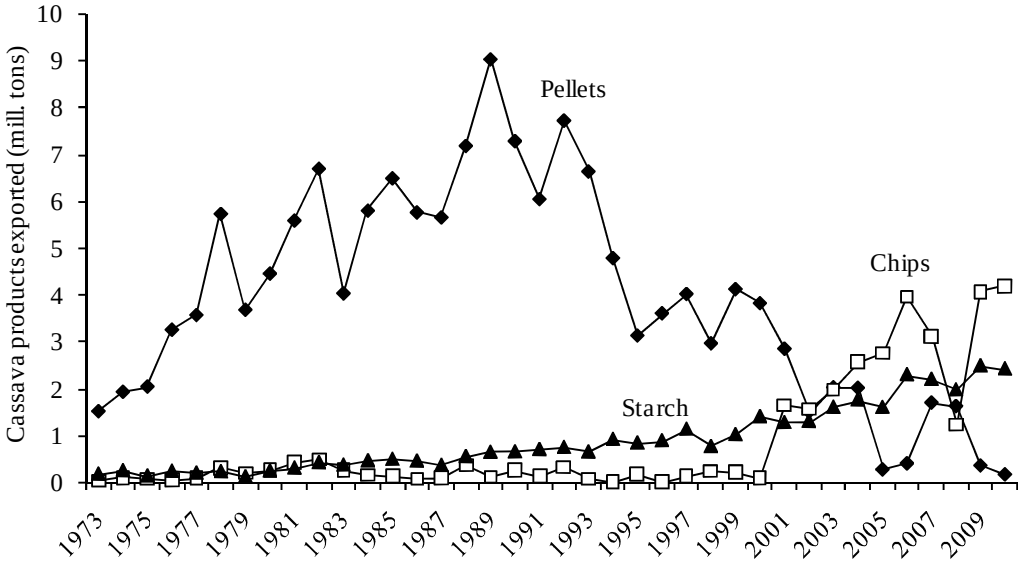

Figure 5. Quantities of cassava products exported from Thailand from 1975 to 2010.
***Source:** Adapted from TTTA, 2006 and 2010.*

3. Pests and Diseases

Until very recently there were no economically important pests or diseases on cassava in Asia – with the exception of India – so there was no need for the use of pesticides. Unfortunately, in late 2008, a new species of mealybug was found in Thailand and later identified as *Phenacoccus manihoti*, a species of mealybug that did not previously exist in Asia. Most likely this species was accidentally introduced into Asia from either Africa or southern Latin America. After the mealybug was identified, the Thai Dept. of Agriculture (DOA) contacted IITA in Benin, Africa, which provided 500 pairs of the well-known parasitoid, *Anagyrus lopezi* (successfully introduced into Africa from southern Latin America in the 1980's to control the rapid spread of the same mealybug there) to Thailand in Sept 2009. This biological control agent is now popularly known as the "lopezi wasp" to control the "pink" mealybug. After intensive initial testing and multiplication by DOA, the wasp was handed over to various research centers and a private company for mass rearing and further distribution. By late 2011 these organizations had produced a total of about 6 million pairs of lopezi wasps. These are gradually being released into the farmers' cassava fields at the rate of about 100-300 pairs per ha. In addition, extension agents and farmers are being trained in the rearing and release of the wasp, and in the soaking of cassava stakes in a solution of 50 ppm of Thaimethoxam for 10 minutes before planting to kill any mealybugs and other insects that may be present on the stakes and to protect the young plant from any mealybug infestation for at least one month. This intensive effort to control the mealybug was prompted by the realization that this insect could seriously affect the whole cassava sector in Thailand, both the approximately 400,000 cassava farmers and the various processing industries. From 2009 to 2010 the cassava harvested area had decreased by about 12% while the yield had decreased 17%, resulting in a decrease in production of 27% or 8.1 million tons of fresh roots, a loss of over 500 million US dollars for farmers, and probably much more than that for the industry.

Table 2. Characteristics of cassava production and utilization practices in eight countries in Asia in 2009.

	Cambodia [1)]	China [2)]	India [3)]	Indonesia [4)]	Lao PDR [5)]	Philippines	Thailand [6)]	Vietnam [7)]
Cassava production ('000 t)	3,497	4,506	9,623	22,039	153	2,044	30,088	8,557
Cassava harv. area ('000 ha)	157	277	280	1,176	10	216	1,327	509
Cassava yield (t/ha)	22.3	16.3	34.4	18.7	14.7	9.5	22.7	16.8
Utilization -main	dry chips -exp/dom	starch -domestic	human consumption	human consuption	human consumption	human consumption	dry chips (50%) -export/domestic	starch exp(70)/dom (30)
-secondary	starch -exp/dom	animal feed ethanol-dom.	starch -domestic	starch -domestic	starch dry chips	starch-dom. ethanol-dom.	starch (50%) -exp(65)/dom (35)	dry chips-export animal feed
Farm size (ha/farm))	2-7	0.7-1.0	0.4-2.0	1.0--3.0	2-3	3-4	3-5	0.8-1.5
Cassava area (ha/farm)	1-4	0.2-0.7	0.2-1.0	0.2-0.5	0.3-0.4	0.2-1.0	2-3	0.2-0.8
Topography	flat-sloping	flat-steep	flat-sloping	sloping/flat	gentle-steep	flat-sloping	flat-sloping	flat-steep
Soil texture	loam-sandy	loam-clay	loam	clay/sandy loam	clayey-loamy	clay-loam	sandy loam	sandy-rocky
Soil fertility	medium-high	medium	low-high	medium/low	medium	medium	low-medium	low-medium
Rainfall (mm)	~1200-1400	~1200-1700	~800-1,400	~1,200-1,600	~1200-1400	~1,200-1,400	~1,200-1,400	~1,200-1,400
Crop. system(%)-monocrop.	95	40	70	40	66	60	95	65
-intercrop.	5	60	30	60	34	40	5	35
Land preparation	tractor	oxen/hoe	hoe/oxen	hoe/oxen	hoe or no-till	hoe/oxen	tractor	oxen/hoe/tractor
Soil preparation	flat or ridges	flat	mounds/flat	ridges/flat	flat	flat/ridges	flat/ridges	flat/ridges
Time of planting	April-May	March	April-May	Oct-Nov	April-June	April-May	Feb-May	March/Sept
Stake planting position	slanted	horizontal	vertical	vertical	horizontal	horizontal	vertical	slanted/vertical
Weeding	by hoe	hoe/herbicides	hoe	hoe	hoe or knife	hoe or knife	hoe/herbicide	hoe
Fertilizer -chemical	38% of farmers	low-very high	medium-high	low (mainly N)	none	none-little	75% of area	~80% of farmers
- organic	little	medium	some	some	some	some	some	medium-high
Irrigation	no	no	no/furrow irr.	no	no	no	no/some drip	no
Harvest	by hand	by hand	by hand	by hand	by hand	by hand	by hand/tractor	by hand
Labor cost (US$/manday) [8)]	2.93	5.80	4.35	3.87	3.50	3.33	4.41	2.50
Labor use (mandays/ha) [8)]	100	75	210	84	78	66	65	125
Production costs (US$/ha) [8)]	965	1,130	1,298	1,455	1,192	881	801	517
Production costs (US$/ton) [8)]	43	69	38	78	81	93	35	31

[1)] El Sotheary, 2010; [2)] Henry and Howeler, 1994; [3)] first entry refers to Kerala, second to Tamil Nadu; [4)] first entry refers to Java, second to Lampung; [5)] Thiphavong Boupa *et al*., 2010; [6)] Office Agric. Economics, 2010; [7)] Pham Van Bien *et al*., 1996; [8)] Howeler, 2010a.

To combat the problem, the Thai government provided about US$ 5 million for the cassava sector, of which US$ 1 million for cassava research by DOA. The effort must have paid of as yields are estimated to have increased again from 18.78 t/ha in 2010 to 20-22 t/ha in 2011.

The same mealybug has also spread to cassava growing areas across the Thai borders with Laos, Cambodia and Myanmar. An intensive effort is under way by FAO, in collaboration with Thai institutions, to train researchers and extension workers in those countries in the effective control of this mealybug.

In practically all countries in Asia cassava is affected by cassava bacterial blight (CBB) disease and *Cercospora* (brown leaf spot and diffuse leaf spot) during the wet season, but these are seldom serious enough to cause a yield decline. Other common disease problems are anthracnose and root rots, which may cause serious yield losses in certain locations. India and Sri Lanka are the only two countries where cassava is affected by a virus disease, the Indian and Sri Lankan Cassava Mosaic Virus (ICMV and SLCMV), which are widespread in India. These two diseases cause serious yield losses in susceptible varieties, but most commercial varieties have a certain degree of tolerance. The virus is spread through infected planting material and by the white fly *Bemisia tabaci*. To combat the problem farmers should use planting material from non-infected mother plants, and should pull out and burn (rouging) any plants showing symptoms of the disease. Recently, tissue culture plants with resistance to ICMD and SLMCV were introduced from CIAT, which are now being used in the breeding program at the Central Tuber Crops Research Institute (CTCRI) to produce locally adapted and high-yielding varieties with a high degree of tolerance or resistance.

Recently another new disease has been causing serious yield losses in South Vietnam, and sporadically in other parts of Vietnam and Thailand. The disease causes excessive proliferation of young buds resulting in small leaves and short internodes; it was identified as being caused by a phytoplasm. This needs urgent and in-depth research to identify the causing agent and ways to combat the problem. Currently, farmers are advised to use only planting material of non-infected mother plants and to remove and burn any plants showing the symptoms.

4. Products and Markets

Both cassava roots and leaves (or young plant tops) have multiple end-uses, including for direct human consumption of fresh roots and leaves (after boiling), on-farm animal feeding, commercial production of animal feed, and production of starch or starch derivatives. **Figure 6** shows in more detail the various products made from cassava starch and dried chips, as well as from the peels and pulp, which are by-products from the starch industry.

a. Fresh roots for human consumption

In Kerala state of India, as well as in some areas of China and Vietnam, fresh cassava roots are consumed directly after boiling or roasting. In most other parts of Asia cassava is not consumed as fresh roots, but only after some form of processing.

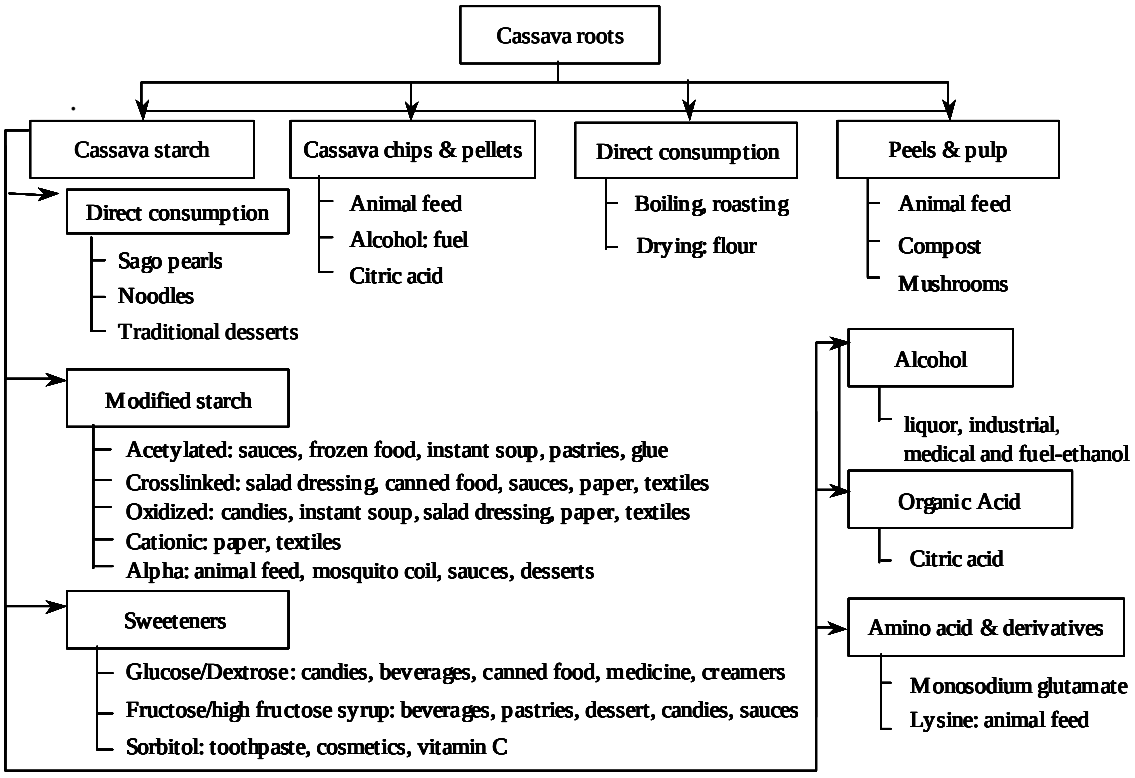

Figure 6. Cassava root processing into value-added products.
***Source**: Adapted from TTFITA, 2000.*

b. Flour for human consumption

The simplest and most common form of processing, used widely in Indonesia, is to peel the roots, wash and slice and then sun-dry for 2-3 days to produce dry cassava chips or chunks, in Indonesia known as *gaplek*. *Gaplek* can be stored and is traded in village markets. When needed, the dry root pieces are pounded into a flour, which is shaken on a bamboo screen with some water to produce granules, called *tiwul*. The size and shape of these granules is similar to rice grains and the *tiwul* is often cooked together with rice to extend the family's limited supply of rice. Presently, small processing plants in Indonesia buy fresh roots to be processed directly into various flour mixes (supplemented with vitamins and flavors) as well as semi-cooked *instant tiwul*. These are mainly destined for urban consumers.

Cassava flour is also used in many baked goods, such as bread, cakes, crackers, icecream cones etc.

c. Dry chips and pellets for animal feed or alcohol

Up until 2002, cassava pellets were the mainstay of the Thai "tapioca" trade, mainly for export to Europe (**Figure 5**).

Fresh cassava roots are taken in small farm trucks from the field to the "chipping yard". These chipping and drying yards consists of a concrete floor, varying in size from about 0.5 ha to as large as 30 ha; they are scattered all through the cassava regions. Using a tractor-mounted front loader, cassava roots are piled up and loaded into large diesel-powered chipping machines. The chipped roots are then spread evenly over the concrete

floor and left there for 2-3 days of sun-drying. The chips are turned every few hours using a rake mounted under a tractor or motor vehicle. When dry (about 14-15% moisture content) the chips are gathered by a tractor with blade and pushed into piles. These dried chips are then taken by truck to the pelleting factories, where the chips are ground up into meal, mixed with a little palm oil and steam and then extruded through a die in the pelleting machine. After cooling, the resulting product consists of small hard sticks, about 2 cm long and 0.5 cm in diameter. These compressed pellets are ideal for long-distance transport, even as far away as Europe. Pelleting reduces the volume (saving transport costs) and the dust, as compared to dried chips. Normally, one ton of fresh roots can produce 450 kg of chips or 440 kg of hard pellets (**Table 3**).

Table 3. Conversion factors for cassava-based products.

1 ton of fresh cassava roots (38% DM) produces:
450 kg of dry chips (85% DM)
440 kg of hard pellets
250-300 kg of native starch
1 ton of dry cassava chips (85% DM) produces:
665 kg of native starch
665 kg of modified starch
665 kg of liquid glucose
770 kg of sorbitol 70%
770 kg of maltol 70%
500 kg of crystal sorbitol
500 kg of mannitol
1 ton of native cassava starch produces:
1,111 kg of sago
1,087 kg of glucose syrup
770 kg of glucose
665-1000 kg of maltose
833 kg of sorbitol
417 kg of MSG
568 kg of ethanol (96%)
1 ton of fresh roots can produce 150-160 liters of ethanol
1 ton of dry cassava chips can produce 350-400 liters of ethanol
1 ton of molasses can produce 250-300 liters of ethanol
1 ton of sugarcane can produce 70-90 kg of ethanol

However, in 2010, Thailand did not export any cassava pellets to Europe, down from 6.0 million tons in 1989. But unlike in 1989 it exported considerable quantities of dry chips, about 4.2 million tons, mostly to China, where it is used for production of commercial animal feed, and alcohol.

Table 4 shows that the export of dry cassava products is still dominated by Thailand, while China is the main importing country, both for cassava chips and starch. Chip imports in China were 4.67 million tons in 2007, but suddenly decreased to 2.00

million tons in 2008, and then rebounded again to 6.12 million tons in 2009. Of this, 4.24 million tons (69%) came from Thailand and 2.01 million tons (31%) from Vietnam.

Table 4. Total world trade in cassava products in 2009.

	Exports ('000 t)					
	Fresh root equivalent	Dry products ('000 t)				
		Starch	Tapioca pearl	Chips+ pellets	Flour	**Total**
World	**19,908**	**1,822**	**46**	**5,412**	**60**	**7,340**
-Americas	333	21	2	104	2	**129**
-Europe	96	14	1	16	0	**31**
-Africa	22	1	0	4	4	**9**
-Asia	**19,450**	**1,786**	**43**	**5,285**	**54**	**7,168**
-Cambodia	0	0	-	0	-	**0**
-China	72	4	14	0	-	**18**
-India	6	0	1	1	0	**2**
-Indonesia	446	13	3	168	0	**184**
-Japan	0	0	0	0	0	**0**
-Korea (ROK)	0	0	0	0	0	**0**
-Malaysia	0	0	0	0	0	**0**
-Philippines	2	0	0	1	0	**1**
-Thailand	17,085	1,743	22	4,357	54	**6,176**
-Vietnam	1,527	-	-	753	-	**707**

	Imports ('000 t)					
	Fresh root equivalent	Dry products ('000 t)				
		Starch	Tapioca pearl	Chips+ pellets	Flour	**Total**
World	**24,782**	**2,062**	**46**	**7,178**	**16**	**9,302**
-Americas	445	61	10	69	2	**142**
-Europe	329	44	3	60	2	**109**
-Africa	174	26	1	25	4	**56**
-Asia	**23,699**	**1,900**	**31**	**7,020**	**9**	**8,960**
-Bangladesh	152	33	**5**	-	-	**38**
-China	18,710	1,198	4	6,1170	0	**7,319**
-India	20	2	3	0	0	**5**
-Indonesia	673	167	0	2	0	**169**
-Japan	478	105	2	21	1	**129**
-Korea (ROK)	1,395	35	0	552	0	**587**
-Lao PDR	9	-	0	-	4	**4**
-Malaysia	680	167	3	0	0	**170**
-Philippines	372	92	1	0	0	**93**
-Thailand	736	0	0	324	0	**324**
-Vietnam	-	-	-	-	-	**-**

Source: *FAOSTAT, Oct 2011.*

Most of the chips and pellets are used for production of alcohol and animal feed, respectively. Besides dry chips, China also imported nearly 1.2 million tons of cassava starch, much of which is being converted to modified starch within China. China itself was producing about 4.5 million tons of fresh roots in 2009 (FAOSTAT, 2011), while importing the equivalent of 18.7 million tons of fresh roots (**Table 4**). Thus, in 2009 about 80% of China's cassava requirements were met from imported dry chips and starch, mostly from Thailand and Vietnam, but also some from Indonesia, Cambodia, Lao PDR and Myanmar. This is driving the current cassava boom in many countries in SE Asia (**Table 5**).

Table 5. Importation of cassava chips from various countries by China in 2009.

Country	Dry cassava chips ('000 t)
Thailand	4,062
Vietnam	2,011
Indonesia	143
Lao PDR	2
Others	0.3
Total	**6,218**

Note: part of the dry chips exported from Thailand and Vietnam was produced in Lao PDR and Cambodia.

***Source**: Boonmee Watanaruangrong, 2010, personal communication*

d. Starch for food and industry

Cassava starch can be divided into *native starch* and *modified starch*. The production of native starch is a relatively simple process, that can be done at many scales, either at the household level, such as in some villages in north Vietnam, Cambodia and on Java island of Indonesia, up to very large and fully-mechanized starch factories, such as those in Thailand, south Vietnam, and in Lampung province of Indonesia. One ton of fresh roots usually results in 250-300 kg of starch (**Table 3**).

During the past decade, the cassava starch industry in Thailand has expanded very rapidly (**Figure 5**), and total production in 2009 was approximately 3.8 million tons consuming about 50% of the total production of 30.1 million tons of cassava roots. Of the 3.8 million tons of starch produced, about 2.5 million tons were exported, of which 1.8 million tons was native starch and 0.7 million tons modified starch, with a value of 537 and 414 million US dollars, respectively. Most of the native starch was exported to China, Taiwan, Indonesia, Malaysia and Japan, while most of the modified starch was exported to Japan, China, Indonesia and Korea.

In Indonesia the cassava starch industry suffered significant losses after the 1997 economic crisis, but has now mostly recuperated. In 2002, total production was 1.34 million tons of starch (P.T. Corinthian, 2004). Practically all cassava starch produced in Indonesia is for the domestic market. In India, most cassava starch is produced in Tamil Nadu (about 90%) and Andhra Pradesh (10%) with a total annual production of cassava starch and tapioca pearls (or sago) of 330,000 tons (Edison, 2001). In China, cassava starch production was about 900,000 tons in 2007, while an additional 500,000 tons of cassava starch were imported (Tian Yinong, 2010). In Vietnam cassava starch production is

increasing rapidly and for 2003 it was estimated at about 500,000 tons, of which 70% was exported (mainly to China, Taiwan and Korea) and 30% used domestically (Hoang Kim, personal communication).

In China the total annual consumption of starch and derived products in 1998 was about 4.03 million tons, of which 3.32 million tons (82.3%) was maize starch, 470,000 tons (11.7%) cassava starch, 96,000 tons (2.4%) each of sweet potato and wheat starch, and 48,000 tons (1.2%) potato starch (Tian Yinong *et al*., 2001). In 2009, China imported about 1.2 million tons of cassava starch, of which 609,000 tons from Thailand. Of the latter, 500,000 tons were native starch and 109,000 tons modified starch. The native starch is used mainly for production of modified starch, sweeteners and MSG.

Tables 6 and **7** show that in 2007 China was the main domestic consumer of cassava products and that over 80% of its requirement of 22.3 million tons of fresh root equivalent was imported from other countries in Asia. Most of the cassava was imported in the form of dry chips (**Table 5**) and used for production of animal feed and other (mostly industrial) uses (**Table 6**). Only about 10% was used for food. In contrast, Indonesia had a similar domestic requirement of about 20 million tons, but about 50% of that was used for food.

Table 6. Production, supply and domestic utilization of cassava in 13 cassava producing countries in Asia in 2007. Data are in fresh root equivalents.

	Domestic supply ('000 t)				Domestic utilization ('000 t)			
Country	Produc-tion	Import	Export	Domestic uses	Food	Feed	Other uses	Waste
Asia	**72,914**	**22,629**	**23,515**	**66,093**	**24,379**	**19,214**	**17,411**	**5,087**
-Thailand	26,916	10	18,404	2,676	883	135	312	1,346
-Indonesia	19,988	1532	991	20,529	9,974	400	7,555	2,600
-India	8,232	6	16	8,222	7,811	-	0	412
-Vietnam	8,193	-	3,762	4,431	623	3,399	-	410
-China	4,362	18,188	318	22,232	2,015	15,068	5,018	131
-Cambodia	2,215	-	12	2,203	364	<1	1,728	111
-Philippines	1,871	161	5	2,027	1,791	75	161	-
-Malaysia	430	610	3	1,036	398	21	595	21
-Lao PDR	233	13	-	126	79	23	-	23
-Sri Lanka	220	22	<1	242	154	55	22	11
-Myanmar	211	2	-	213	192	-	-	21
-East Timor	41	-	-	41	40	-	-	1
-Bangladesh	-	235	-	235	29	-	206	-

[1)] Much of the "waste" (peels, solid residue from starch extraction etc.) is used for industrial purposes or animal feed.

Source: *FAOSTAT, Commodity Balances, July 2010.*

In India, East Timor, Myanmar and the Philippines practically all cassava was domestically produced and used for human consumption, mostly after boiling of fresh roots, or in the form of processed products such as sago, starch and a variety of snack foods. In Vietnam cassava is mainly used for animal feeding, either on-farm or in

commercial animal feed rations, but much cassava is also used for starch production, which is not shown in **Table 6**.

Table 7. Total domestic food supply (in fresh root equivalents, '000 t) and utilization (%) of cassava, as well as the per capita supply as food and its contribution to the diet in 13 cassava producing countries in Asia in 2007.

		Domestic utilization (%)				Total food supply ('000 t)	Per capita food supply			
Country	Popula-tion (mil.)	Food	Feed	Other uses	Waste		Fresh equiv. (kg/yr)	Energy (kcal/d)	Protein (g/day)	Fat (g/day)
Asia (13)	**3,108**	**36.9**	**29.1**	**26.3**	**7.7**	**24,379**	**6.15**	**16.15**	**0.09**	**0.04**
-Thailand	63	33.0	5.0	11.7	50.3	888	13.18	39.89	0.31	0.07
-Indonesia	223	48.6	1.9	36.8	12.7	9,974	44.39	121.67	0.70	0.29
-India	1,081	95.0	-	-	5.0	7,811	6.71	15.19	0.07	0.02
-Vietnam	82	14.0	76.7	-	9.3	622	7.23	20.01	0.14	0.06
-China	1,313	9.1	67.8	22.5	0.6	2,014	1.51	4.44	0.04	0.01
-Cambodia	14	16.5	0.1	78.4	5.0	364	25.43	70.37	0.49	0.21
-Philippines	81	88.4	3.7	7.9	-	1,791	20.19	55.85	0.39	0.17
-Malaysia	25	38.5	2.0	57.5	2.0	398	14.99	40.86	0.29	0.11
-Lao PDR	6	63.2	18.4	-	18.4	79	13.04	35.63	0.23	0.10
-Sri Lanka	19	63.6	22.7	9.1	4.6	154	7.74	32.45	0.15	0.04
-Myanmar	50	90.1	-	-	9.9	192	3.91	10.81	0.08	0.03
-East Timor	<1	97.6	-	-	2.4	40	37.57	91.36	0.38	0.15
-Bangladesh	150	12.3	-	87.7	-	29	0.19	0.46	0.00	0.00

[1)] much of the "waste" (peels, solid residue from starch extraction etc.) is used for industrial purposes or animal feed.

Source: *FAOSTAT, Food Supply, July 2010.*

Table 7 shows that per capita consumption of cassava-based foods was highest in Indonesia, followed by East Timor, Cambodia and the Philippines. It is an important source of calories, especially for the poorer and rural segments of the population, while it is an important ingredient in many snack foods consumed by the general population of Indonesia. While Thailand is the largest producer of cassava in Asia, only about 10% is used domestically, mainly for production of food, commercial animal feed and various industrial (non-food) products such as modified starch for the paper and textile industries, and recently for production of fuel-ethanol (**Table 8**). Thai data show up to 30% domestic use of cassava in the country, much higher than the FAO data in **Table 7**.

e. Modified starch

Native starch can be modified by either physical, chemical or enzymatic processes, producing different forms of "modified" starch with distinctly different properties and different uses. Modified starches are used in many different types of foods as well as in industry, mainly for production of high quality paper, for textile sizing and some animal

feeds (**Figure 6**). One of the main users of modified starch is the paper industry. Cationic starches made from cassava starch are particularly suitable for the sizing and coating of paper in high-speed paper making machines (Jin Shuren, 2001). Other main users of modified starch are in the food industry, textiles, in agriculture and in animal feed, while smaller amounts are used in construction materials, in casting, oil drilling and medicines.

f. Starch-based sweeteners

Cassava starch can be used for the production of many types of sweeteners after hydrolyzation by either acids or enzymes, or both. These sweeteners include maltose, glucose syrup, glucose and fructose, which can be further processed into various oligo-saccharides (Jin Shuren, 2001).

g. Hydrogenated sweeteners.

These include sorbitol, mannitol and maltol. They are produced by treating starch with hydrogen gas in high-pressure tanks, using a special catalyst and ion-exchange resins. Sorbitol is used mainly for the production of vitamin C and as a moisture conditioner in toothpastes (Jin Shuren, 2000).

h. Organic acids

Organic acids made from cassava starch include citric acid, acetic acid, lactic acid and itaconic acid, which are used in the food industry as well as for the production of plastics, synthetic resins, rubber products etc. Lactic acid is produced by the fermentation of starch with *Lactobacillus amylovorus* (Wang Xiaodong *et al*., 1997; 2000).

i. Monosodium glutamate (MSG) and lysine

MSG is a well-known flavor-enhancing agent used in many Asian kitchens. It is made through the microbial fermentation of starch or sugar (molasses) in the presence of ammonium salts. In Thailand, MSG production is one of the main consumers of native cassava starch (**Table 8**). Lysine is an important amino-acid used as a supplement in animal feed, especially for pigs.

Table 8. Estimated use of cassava starch in various industries in Thailand in 2007.

Industries or products	Starch usage ('000 t)	Starch usage (%)
Glucose, high fructose, sorbitol	460	35.4
Monosodium glutamate	250	19.2
Food factories and cooking	200	15.4
Modified starch	150	11.6
Paper	120	9.2
Tapioca pearls (sago)	60	4.6
Textiles	10	0.8
Others (glue, medicine, plywood)	50	3.8
Total	**1,300**	**100**

***Source**: Office of Agricultural Economics, 2008.*

i. Degradable plastics

Various types of starches are being used for the production of bio- or photo-degradable plastics, either by mixing starch or modified starch with polyvinyl hydrocarbons, or by polymerization of starch, which is then blended with various other polymers (Sriroth *et al*., 2001). The use of cassava starch for these processes still requires much research

k. Ethanol

Currently, many countries in Asia use cassava as the feedstock for the production of ethanol; this includes drinking alcohol, industrial alcohol and fuel-alcohol. The latter is also called "dehydrated" or "anhydrous" ethanol and must be 99.5% pure ethanol.

In the late 1970s several ethanol distilling factories were set up in Brazil using fresh cassava roots as raw material. The ethanol was used as automotive fuel, either mixed with gasoline (up to 20% alcohol) for which no motor modification is required, or as pure anhydrous ethanol, in which case the carburator and some other parts need to be modified (de Souza Lima, 1980). Both result in less atmospheric pollution than the use of 100% gasoline. The use of cassava for production of fuel-ethanol was later discontinued in Brazil in favor of sugarcane, as the bagasse from sugarcane can supply much of the energy needed in the production process.

In China, several factories in Guangxi are now using the solid waste (pulp) of the cassava starch industry for the production of ethanol (Gu Bi and Ye Guozhen, 2000). Other alcohol factories in China are switching from the use of molasses to that of cassava chips for alcohol production, because of strict pollution control requirements that makes the use of molasses uneconomical. In Guangxi there are now about 200 alcohol factories, most of which still use molasses as the raw material. But about 20 factories use mainly cassava fresh roots, supplemented with cassava dry chips and molasses when no fresh roots are available. These produce about 20,000-30,000 tons of hydrous ethanol (95% ethanol) per year, mainly for export or industrial use.

Since about 2002 the Chinese government has promoted the use of "gasohol" instead of gasoline, in order to reduce the importation of oil and reduce air pollution from greenhouse gasses. There are presently four large companies producing anhydrous or fuel-ethanol in four provinces, mostly located in the north and northeast. Three of these use maize and one uses wheat as the raw material. Together they produce about 1 million tons of fuel-ethanol per year, or 3.35 million liters per day. Since maize and wheat can be better utilized as food or animal feed, the Chinese government is planning to phase out the use of these crops for production of fuel-ethanol. Instead, they want to promote the use of sweet sorghum in the northern provinces and cassava in the south. Thus, in the southern provinces of Guangxi, Guangdong, Hainan and Yunnan, major investments are now being made in the construction of large factories to produce anhydrous ethanol for the production of "gasohol E10", i.e. 10% ethanol mixed with 90% gasoline. One factory located in Beihai is producing about 840,000 liters of fuel ethanol per day from cassava, while at least two others are under construction or in the planning phase (**Table 9**).

In Thailand "gasohol", containing 10% ethanol, is presently available in most gas stations and this has become a popular fuel because of its lower price ($0.12-0.13/liter lower than gasoline). Initially, almost all ethanol was made from molasses, but recently

many factories have been completed or are under construction that will use fresh or dried cassava, or can use either cassava or molasses, depending on the price of raw materials. By the end of 2011 there will be nine factories that use cassava or cassava/molasses as the raw material, while another factory will finish construction in 2012 (**Table 9**).

Table 9. Actual or planned factories for the production of anhydrous ethanol from cassava in Asia (Oct 2011).

Country/Company	Location	Capacity ('000 l/day)	Date completed	Fresh root requirement ('000 t/year)[1)]
Cambodia				
MH Bio-Energy Co.	Kandal	144	2008	270
China				
China Food Comp (COFCO)	Beihai, Guangxi	840	2007	1,575
China Food Comp (COFCO)	Wuzhou, Guangxi	1,260	planned	2,362
Other Company	Longan, Guangxi	420	planned	787
		2,520		4,724
Indonesia				
Medco	Lampung	200	2009	375
Indonesia Ethanol		167		312
EN 3 Green Energy	South Sulawesi	600	2013	1,125
		967		1,812
Thailand				
Thai Nguan Ethanol Co.	Khon Kaen	130	Aug 2005	244
Ratchaburi Ethanol	Ratchaburi	150	Jan 2009	281
Supthip	Lopburi	200	May 2009	375
Taiping Ethanol	Sra Kaew	150	July 2009	281
PSB Starch Production	Chonburi	150	Aug, 2009	281
Sima Inter Products	Chachoengsao	150	Dec 2011	281
Thai Agro Energy	Suphanburi	200	Dec 2011	375
Double A Ethanol	Prachinburi	250	Dec 2011	469
PTK Ethanol (phase 1)	Nakhon Ratchasima	340	Dec 2011	638
Impress Technology	Chachoengsao	200	2012	375
PTK Ethanol (phase 2)	Nakhon Ratchasima	680	after phase 1	1,275
		2,600		4,875
Vietnam				
Petrosetco+Itochu Co.	Phu Tho	333	Dec 2011	624
Petrosetco+Itochu Co.	Binh Phuoc	333	Mar 2012	624
Petrosetco+Itochu Co.	Quang Ngai	333	Mar 2012	624
		999		1,872

[1)] based on 300 working days per year and a conversion of 160 l ethanol/ t fresh roots

The total capacity of these cassava-based factories will be 2.750 million liters per day. In addition, there are also 13 factories using molasses or sugarcane juice with a total capacity of 2.445 million liters per day. However, these factories were operating at only about 40% of capacity in 2010 because of the exceptionally high cassava price and lack of

domestic demand. When all fuel ethanol plants that are currently under construction are completed by the end of 2012, Thailand will have an installed capacity to produce 5.195 million liters of ethanol per day, or 1,013 million liters per year. Much of this will be destined for export. In 2010 Thailand exported 48 million liters of fuel ethanol, while up to Aug 2011 this had already reached 83 million liters (DEDE, 2011). When all these cassava-based factories are working at full capacity, these will require about 4.87 million tons of fresh roots, or about 16% of current production (**Table 9**).

The Thai Ministry of Energy is planning to increase markedly the number and capacity of cassava-based ethanol factories. While the factories operating in 2010 required about 2.27 million tons of fresh roots, in 2022 this may increase to 16.43 million tons Unless cassava yields can be markedly improved there will continue to be a shortage of supply as the cassava growing area is not likely to increase, and may actually decrease during the next ten years due to urbanization and competition for land from other crops. The Thai government may encourage the use of cassava for the production and export of ethanol and other value-added products, and restrict the export of less valuable products like dry chips and pellets. **Figure 7** shows that this could markedly reduce the export of cassava chips from Thailand to other countries, especially to China, which in turn may stimulate more cassava production in neighboring countries in Asia.

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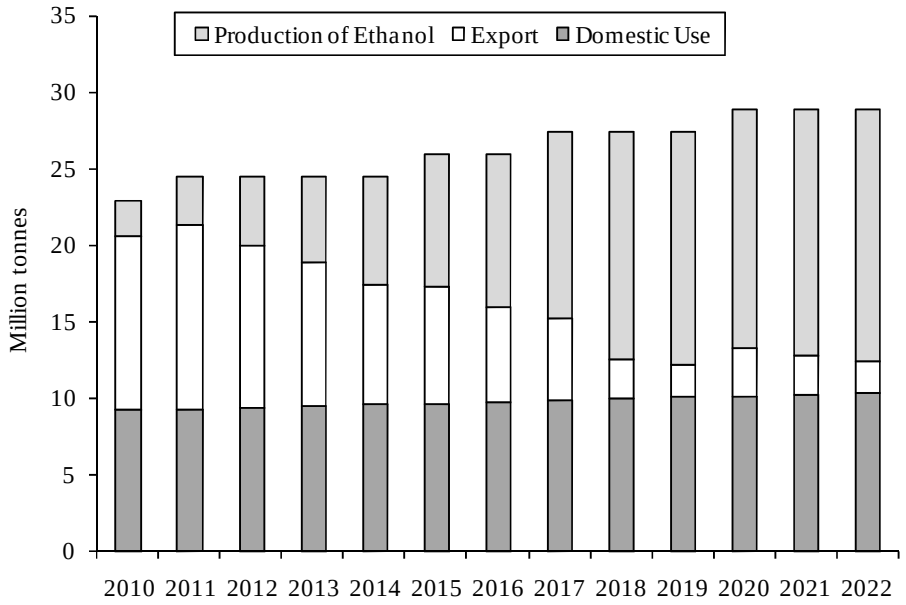

Figure 7. Estimated use of fresh cassava roots in Thailand for domestic use, ethanol production and export from 2010 to 2022.
***Source**: Dept. of Alternative Energy Development and Efficiency; www.dede.go.th*

Several other countries in Asia have constructed, or are in the process of constructing, cassava-based fuel ethanol factories, and more are likely to follow as many

countries have now established mandates to partially replace pure gasoline with gasohol containing 10, 20 or even 85% dehydrated ethanol in the next 10-20 years.

MAINTAINING A COMPETITIVE EDGE

To keep cassava-based products competitive in domestic as well as world markets is a real challenge. While cassava has many favorable attributes in the area of production, it also has some negative attributes, especially in terms of post-harvest handling due to its high water content and rapid deterioration. The content of cyanogenic glucosides in the roots is an important consideration in the use of cassava for direct human consumption, but is of less importance for production of processed food, animal feed or starch. The low content of protein in cassava roots increases the efficiency of starch extraction, but also means the absence of a valuable high-protein by-product, as is the case for maize starch.

Finally, since cassava cannot be grown in temperate climates, it has never received the same research attention in developed countries as for instance maize, rice, wheat, soybean and potato. Research on cassava had been minimal until the early 1970s when the international research centers – CIAT in Colombia and IITA in Nigeria – received the mandate for cassava research and development, which in turn triggered the formation of many national cassava research programs. Nevertheless, the number of researchers working on cassava, and the research budgets dedicated to this crop, are minimal in comparison with those for most of the competing crops.

Still, cassava thrives in Asia because of the ability of farmers, processors, traders, researchers and policy makers to adapt to rapidly changing physical, biological, economic and social conditions. To maintain this competitive edge will require special attention in three areas: 1) improving the production system in order to reduce the cost of raw material while maintaining reasonable profit margins for farmers; 2) adding post-harvest value by the development of new products and more efficient processes; and 3) stimulating higher demand for cassava-based products by market development. While the development of new markets was an important activity a few years ago, it now seems less urgent as demand for cassava in Asia seems to far outstrip supply. With the rapidly increasing use of cassava as a renewable energy source to replace fossil fuels both for production of ethanol in the transport sector and for production of many chemicals, such as biodegradable and non-degradable plastics (Samai Jai-In *et al*., 2010) the demand for cassava roots are expected to remain very high in the near future.

While this cassava “boom” in Asia is a welcome development, which is likely to benefit many cassava farmers and improve their livelihoods, it may also stimulate a rush to expand cassava planting to less suitable areas, especially to steep slopes, which may cause serious erosion and soil degradation, or trigger further and more rapid deforestation in those areas where land is still available. To prevent this long-term detrimental effect on land and forest resources, it is essential to increase investments in cassava research both at the national and international level so as to obtain increases in production without having to increase the planting area. This will require that governments start considering cassava as a strategically important crop, similar to rice, maize, rubber and sugarcane, and markedly

increase investments in research in cassava breeding, soil fertility management and erosion control, as well as in integrated pest and disease management, coupled with increased efforts in farmer participatory research and extension.

Cassava yields in Asia have increased more than in other continents mainly by the widespread adoption of higher yielding varieties, which in turn responded to improved crop management practices. This widespread adoption was achieved through the close and effective collaboration between national research and extension institutions working together with local and provincial government officials. The use of farmer participatory research (FPR) and extension (FPE) methodologies, in which farmers become directly involved in the testing, selection and dissemination of new technologies, played a major role in enhancing the adoption of these technologies (Howeler, 2010b). This participatory approach need to be further developed and become part of the institutional culture. Moreover, the active collaboration between various institutions within each country need to be strengthened, and an effective partnership between the public and private sector need to be created if we want to maintain cassava's competitive edge in world markets, while helping farmers to improve their livelihood and maintain our natural resources for future generations.

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CHAPTER 2

CASSAVA: A BASIC ENERGY SOURCE IN THE TROPICS

James H. Cock[1]

INTRODUCTION

This paper on cassava as a basic energy source revisits an analysis of the world cassava situation just over a quarter of a century ago (Cock, 1982; 1985). Those points which stand and are valid are left much as they were, whilst those aspects which have changed markedly or where radical new information has become available are highlighted.

The Cassava Crop

Cassava (*Manihot esculenta Crantz*) is a perennial vegetatively propagated shrub grown throughout the lowland tropics for its starchy, thickened roots. The fresh roots of cassava contain 30 to 40 percent dry matter and have a starch content that approximates 85 percent of the dry matter. In developed countries, where it is a foodstuff of minor importance, cassava is commonly known only in the forms of tapioca, starch pearls or flakes, or as a component of animal rations. In developing countries, however, it is a major food staple. In the 1980s, after rice, maize, and sugarcane, cassava was the fourth most important dietary source of calories produced within the tropics and it probably still holds that position due to its great importance in the diet in Africa. In the twenty first century there are only limited areas of Asia and the neo-tropics where it is the major source of calories for large segments of the population. On the other hand, in Africa cassava is the single most important source of dietary energy for a large proportion of the population living in the tropical areas. Sufficient cassava is consumed as food to provide one billion people with 20% of their dietary energy requirement, and more than 700 million people are highly dependent on cassava as a food.

Cassava has long been a basic staple. There is direct evidence of its cultivation 2500 years ago and circumstantial evidence that the crop may have been cultivated for 6000 years in the Americas (Allem, 2002). It has been suggested that many areas now under tropical rainforest were once cultivated with cassava and maize in shifting culture. On the arrival of the conquistadores from the Old World, cassava was found throughout the lowland tropics of the Americas and the Caribbean. The cassava was either eaten after boiling or was rasped, after which the toxic juices were eliminated by squeezing the mash in basket-weave tubes (known as a tipiti in Brazil) and the remaining mash was roasted to a meal. Cassava production appears to have decreased after the arrival of the conquistadores, when the population of many lowland areas was decimated by introduced diseases. With the opening up of trade between Africa and Brazil by the Portuguese, cassava was taken to the Congo Basin in the 16th century. Two centuries later the crop was independently introduced to Madagascar and the east coast of Africa from where it was taken inland and

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rapidly became established as a basic food staple (Jones, 1952). The introduction of cassava to Asia is not well documented, but the plant was probably taken to the Philippines in the Manila galleon from Acapulco, Mexico, in the 17th century. It was already grown in Indonesia by 1740, and it was probably introduced to Goa by the Portuguese somewhat earlier. By the end of the 19th century the crop was dispersed throughout lowland tropical Asia and the islands of Oceania.

Cassava production has continued to expand throughout the lowland tropics, mainly on the less-fertile, poor-quality agricultural lands. In Africa the capacity of cassava to grow and yield well on low-fertility soils, its ability to withstand locust attacks and drought, and its low cost of production have provided the economic incentive to use it as a replacement for other traditional root crops such as yams. In areas of Africa where population growth has caused a reduction of the rotation pattern in shifting culture and a commensurate decline in soil fertility, cassava is one of the few crops that can still be successfully grown, provided some form of rotation remains. Similarly, in southern India and Java, as the population increased, cassava was increasingly grown as a basic dietary staple on low-quality land that is not suitable for rice. However, in Asia the major growth of cassava has been not as a direct human food but as a low-cost source of energy in animal feed and as a source of starch for food and industrial uses.

Cassava Production

World cassava production for 2007 was estimated at 228 million tons, with 118 million tons in Africa, 72 million tons in Asia, and 37 million tons in South America (FAOSTAT, 2008) (**Figure 1**). This is the energy equivalent of 80 to 100 million t cereal grain equivalent. The area harvested was 18.6 million hectares, with a mean yield of 12.2 tons per hectare (equivalent to 4 to 5 tons of grain per hectare). During the last 25 years, total cassava production has increased markedly due to both increases in area planted and a substantial increase in yield of about one third in the world, and a marked increase of 50% in Asia. This contrasts with the previous quarter of a century in which production increases were largely due to an increase in area planted with yields essentially stagnant. This change is largely based on the large research efforts initiated on cassava in the 1970s, which are now bearing fruits. The average yield of 12.2 tons per hectare is far below the maximum experimental yields of 80 tons per hectare in a 12-month growing season. However, since much cassava is grown with little or no use of fertilizers, fungicides, insecticides, herbicides and irrigation, these yields of 4-5 t grain equivalent per ha compare favorably with the yields of other basic energy crops such as the cereals. Although two grain crops can be harvested each year in some tropical areas, this is not possible in regions where there is a long dry season and irrigation is not feasible. In these regions, where cassava is frequently grown, only one cereal crop can be harvested and with traditional low input management cereal yields are only 1 to 2 tons per hectare per year.

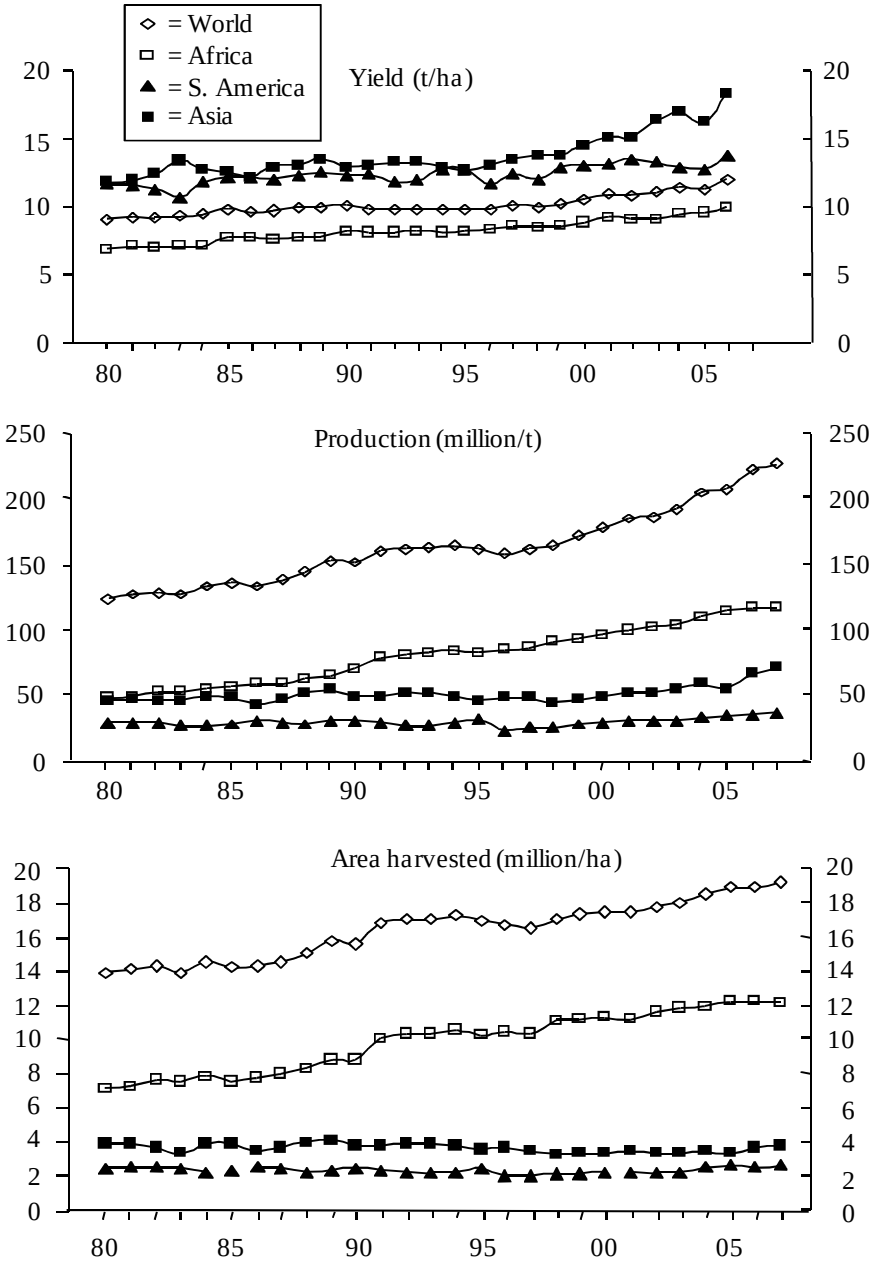

Figure 1. Production, yield and area harvested of cassava.
***Source**: FAOSTAT, 2008.*

Cassava is produced mainly by small-holder farmers, although there are a few large plantations. Small-holder farmers generally follow agronomic practices that do not depend on inputs normally associated with modern agriculture. Planting material is derived from the stems of mature plants. When cut into “stakes” or “cuttings” these will sprout axillary buds 2 to 3 weeks after being placed in the soil. The plant grows, becoming well established after 3 to 4 months when it begins to produce thickened roots. The roots are generally harvested any time from 7 to 18 months after planting. In some areas, cassava is grown as a famine reserve crop, and the plants are left until required. Roots are harvested by pulling on the stem until the whole plant is uprooted.

Cassava Consumption

Somewhat surprising it is more difficult today than 25 years ago to determine the end use of the cassava being produced. Lynam (2008) has estimated consumption patterns **(Table 1)**. In Africa, consumption of cassava as food continues to be high with many people dependent on cassava as their major calorie source. There are reports of chronic cyanide toxicity in regions of Africa where cassava consumption levels are high (Rosling, 1987). Cyanide is liberated from root tissues when they are damaged, by the action of linamarase on linamarin. Cassava clones that have high cyanide contents, and which are normally bitter to the taste, can cause acute cyanide poisoning if the roots are eaten without being processed. This type of poisoning is rare, however, and it is the long-term ingestion of low levels of cyanide from cassava that has more commonly been associated with goiter, cretinism, tropical ataxic neuropathy, and tropical diabetes. Cyanide is detoxified by the formation of thiocyanate from thiosulfate. Thiosulfate is formed from sulfur-containing amino acids, the presence of which is essential for detoxification. Tropical ataxic neuropathy is associated with protein malnutrition and extremely low levels of sulfur amino acids in the blood. Thiocyanate inhibits thyroid uptake and iodine transport and is, thus, associated with goiter and cretinism. Problems associated with cassava toxicity are not widespread outside Africa, however, and occur only in areas where processing of the roots is rudimentary, dietary iodine levels are low, and the intake of protein and sulfur amino acids is suboptimal (Rosling, 1987). Chronic cyanide toxicity has not been reported in Kerala, southern India, where people at one time consumed more than 700 kcal/day as cassava. Protein consumption in Kerala was low (37.8 to 41.5 grams per person per day), but the amino acid content of the protein is reasonably well balanced, with fish being a major component (United Nations, 1975; Kumar, 1979). Similarly, Tukanoan Amerindians consume massive amounts of well processed cassava and show none of the symptoms of cassava toxicity found in African populations (Dufour, 1992). This suggests that chronic cyanide toxicity need not occur when overall processing and nutrition is adequate. The major problems with chronic cyanide toxicity occur in periods of famine or general food shortage. As food becomes scarce the population turns to their famine reserve, which is frequently cassava. In their haste to obtain food, the often rudimentary food processing is curtailed or incomplete, increasing the residual levels of cyanide in the food (Rosling, 1987). At the same time, in a period of famine the consumption of proteins and vitamins tends to decrease. Thus, in periods of famine the population becomes dependent on a diet based on inadequately processed cassava without the accompanying supplements that in more normal circumstances reduce the risk of chronic cassava toxicity.

Table 1. Consumption of cassava as percentage of total for each region.

Region	Food	Feed	Export	Other
Africa	91	8	0	1
Asia	50	8	32	10
S. America	43	51	1	5

***Source**: Lynam, 2008.*

The amount of cyanide in cassava can be greatly reduced by adequate processing. In areas of northeast Brazil, large amounts of *farinha* (a type of cassava flour) are consumed. During *farinha* production most of the cyanide is eliminated when the cassava mash is squeezed and the water, containing much of the cyanide, is discarded. More cyanide is eliminated when the resulting mash is roasted. There is no evidence of chronic cyanide toxicity among the consumers of *farinha*. . Many of the African products, such as gari, are processed using variations on the *farinha* theme. It is safe to say that with adequate processing, the cyanide level of cassava roots can readily be reduced to levels where it is not a problem

Although cassava is of somewhat low nutritional value, it is, at least in the dried form, among the least expensive available sources of calories. While it is true that cassava is not a complete food, calorie deficiency is widespread in the developing countries. Current estimates suggest that 900 million people are under- or mal-nourished; this is about 12% of the world population. Cassava, due to its particular characteristics, has a major role to play in improving the nutrition of the poorer and most undernourished populations of the developing countries.

Biological Potential

Most cassava is grown between 30^{o}N and 30^{o}S, in areas where annual rainfall is greater than 750 millimeters and annual mean temperature is greater than 18^{o}C to 20^{o}C. Small amounts of cassava are grown near the equator in South America and Africa at altitudes up to 2000 meters, under annual mean temperatures as low as 16^{o} to 17^{o}C, but with minimal seasonal fluctuations. Cassava is potentially one of the most efficient crops in terms of starch production. Yields of 80 tons of fresh roots per hectare per year (29 tons of dry roots per hectare per year) have been obtained under optimum growing conditions but without supplementary irrigation (de Vries *et al*., 1967). In areas with high rainfall, total radiation is reduced by cloud cover and yields of 30 tons of dry roots per hectare per year appear to be close to the theoretical yield limit. Several other crops, such as sugarcane, maize, sorghum, and rice have yield potentials of a similar order when one, two, or three crops are harvested per year; hence, in these situations, cassava has no great advantage over other crops.

The yield potential of cassava is not based on a particularly high total biomass production. The genus *Manihot* appears to be in an evolutionary stage somewhere between C3 and C4 photosynthetic pathway (El Sharkawy *et al.*, 2008). Neither the photosynthetic rate of individual leaves nor the maximum crop growth rates for cassava are high. The maximum recorded levels of these parameters are, in fact, much lower than the highest

recorded rates for other major crops such as sorghum, maize, and sugarcane. Thus, the yield potential of cassava and its comparative advantage as a crop are not related to a capacity to produce large amounts of biomass.

Cassava has a relatively long, 9-month to 2-year growing season, and a remarkably high harvest index (ratio of weight of economically useful parts to total biomass production). It is these two factors that enable cassava to produce yields similar to, or greater than, other major crops under optimum conditions. It is, however, under suboptimal conditions that the yield potential of cassava excels when compared with other crops. Crops grown in many tropical areas suffer from uncertain rainfall, long dry periods, and soils with low pH, high aluminum concentrations, and low fertility. In the 1960s, the strategy of the Green Revolution to increase agricultural production was largely directed at removing these constraints through the use of irrigation, soil amendments, and fertilizer applications, and by combining the improved agricultural conditions with plant varieties capable of exploiting them. Since those halcyon days, high energy costs and environmental concerns have made it necessary to search for crops and farming systems that are *per se* tolerant of adverse conditions and that have the capacity for an acceptable degree of productivity under a regimen of low inputs. The characteristics of cassava are in line with this new perspective.

When a cassava crop encounters an environment with limited available resources, particularly nutrients and water, it utilizes those resources extremely efficiently to produce the economically useful part of the plant, the roots. Early work at the University of Queensland, indicated that for maximum growth, cassava's requirements for nitrogen, potassium, and calcium are similar to other crops, but its phosphorus requirements in nutrient solution or sterilized soil are considerably higher (Jintakanon *et al.*, 1982; Howeler, 1980). However, with the exception of phosphorus, the reduction in growth at low nutrient levels is much less in cassava than in other crops, suggesting that the crop is highly tolerant of low nutrient levels. Furthermore, in soils, as opposed to nutrient solutions, where mycorrhizal infection occurs, the phosphorus requirements of cassava are also low. Whereas most crops when faced with low soil nutrient levels continue producing leaves and leaf area, cassava tends to produce a total leaf area commensurate with maintaining high levels of nutrients in the leaves (see Chapter 3). This strategy is theoretically more efficient than that of producing more leaf area but with diluted nutrient content. Thus, under natural conditions with low nutrient availability, cassava can yield nearer its potential total biomass than most other food crops. This picture looks even brighter when economic yield is considered. Under nutrient stress the proportion of total dry matter production diverted to the roots is greatly increased, particularly in more vigorous clones (see Chapter 3). The reduction in starch yield of vigorous clones under nutrient stress is much less, proportionately, than the reduction in total biomass production. In anthropomorphic terms, it can be said that when cassava is under a tight budget system, it spends very wisely.

The tendency of cassava to increase the distribution of biomass to the roots also occurs under water stress. This effect can be so marked that vigorous clones may yield more under stressed than under non-stressed conditions (see Chapter 3). During drought stress cassava follows a conservative pattern of water use, closing its stomata and reducing

the formation of new leaves (see Chapter 3). The stomata remain open only at those times when the evaporative demand is low and the water use efficiency is greatest. Heliotropic responses of the leaves lead to maximal light interception at these times (Berg *et al.*, 1986). In addition, leaf drooping or folding mechanisms protect the leaves at midday from damage due to excessive radiation load (Catalayud *et al.*, 2000). The leaves that remain on the plant during a drought also recover rapidly and are able to actively photosynthesize when moisture becomes available again (see Chapter 3). Thus, the plant slows its growth during drought periods, but rapidly recovers when they cease. These remarkable mechanisms make the cassava crop exceptionally water use efficient. We have estimated that the water use efficiency of cassava in terms of starch produced per unit of water is similar to, or greater than, that of even the most renowned drought resistant crops such as sorghum (El Sharkawy and Cock, 1986; El Sharkawy, 2006).

Unlike many other crops, cassava, once established, has no critical period when drought or other stresses will cause a disastrous decrease in yield. Hence, cassava is well adapted to areas that experience a long dry season or uncertain rainfall. It is extremely rare to find famine caused by uncertain rainfall patterns in areas where much cassava is grown: many farmers and communities in the dry hinterlands of Northeast Brazil rely on cassava to carry them through the drought years.

In traditional growing areas, the native cassava clones tend to be resistant to the disease and pest complexes of the region. For example, clones from the eastern plains of Colombia are usually resistant to cassava bacterial blight, superelongation disease and anthracnose, which are endemic in this area, but are susceptible to phoma leaf spot which is only found in the highland areas. As with many other food crops, when cassava is introduced to new areas, with initially low disease and pest pressures, diseases and pests that flourish in that environment may subsequently be introduced and cause severe yield losses. This has undoubtedly occurred in the cases of the green spider mite in East Africa and mealy bugs in West Africa, where the introduced pests have caused great reductions in yields. Nevertheless, in the Americas it appears that over the centuries farmers have selected clones that are highly resistant to the disease and pest complexes prevalent in their cassava growing areas.

Breeding for Increased Yield

The ability of cassava to survive low inputs and water stress and its demonstrated resistance to pests and diseases make this crop a leading candidate for low-input agricultural systems. Nevertheless, world mean yields for cassava are far below the yield potential. A major question is whether it is possible, through breeding, to obtain clones that are able to approach the demonstrated yield potential and maintain it over a number of years, even under marginal conditions. Farmers have already selected lines of cassava well adapted to local cassava-growing conditions, but they probably have not exploited the true yield potential of the crop. Furthermore, in the traditional slash-and-burn culture, where many of the original farmer varieties originated, cassava is normally widely spaced and planted with other crops. Under these conditions selection may well be for yield per plant rather than for yield per hectare. Yield per plant of segregating populations may be negatively related to yield per unit area under dense planting and good growth conditions

(Kawano *et al.*, 1978, Kawano, 2003). Furthermore, the material available to farmers to select under natural conditions will be from the chance seedlings that occur in the field. The majority of these seedlings will be the progeny of early flowering highly branched types; these types are not optimal for production in dense stands. Hence, it would appear that there are opportunities for developing improved varieties well adapted to the varied conditions where cassava is grown. There is no doubt that the increased productivity over the past quarter of a century in Asia is largely due to the availability of improved higher yielding varieties.

Established breeding programs outside of the neo-tropics had limited access to germplasm and relatively little variability to exploit until the 1970s (Chareinsak, 1988).This situation radically changed with the collection of germplasm assembled at CIAT from about 1969, and its massive but safe distribution to various breeding programs, primarily in the form of sexual seed, particularly in the last twenty five years of the last century. This exchange was probably of particular importance in distributing drought tolerant materials and may in the future be needed to improve materials for highland areas far from the center of origin. Much of the germplasm originally distributed around the world was the result of exchange from the humid coastal regions of Brazil. The Brazilian coastal areas are more humid than the hinterlands, particularly in the NE of Brazil, and it is likely that most of the materials transported were better adapted to humid conditions. The major modern breeding programs led by IITA in Africa, CTCRI in India, CNPMF and IAC in Brazil, Rayong station and Kasetsart University in Thailand and CIAT, have made a major contribution to making available improved disease resistant, high dry matter varieties for the lowland tropical regions. These improved varieties are now widely grown and the increased yields of cassava on a world basis are, at least partially, due to the increased area planted to improved varieties.

From the inception of most of these breeding programs there was a major philosophical difference between their approach and that of the green revolution: the new cassava varieties should be well adapted to the specific conditions where they were to be grown, rather than trying to mould the conditions to suit the needs of the varieties (Kawano and Cock, 2005). This would be expected to have led to individual varieties being selected for particular sites and conditions, and to a certain extent this has occurred. Thus, for example, the IAC varieties and those of CTCRI are restricted to the areas where they were bred and selected. On the other hand, the Rayong and Kasetsart varieties have been successful over a wide range of geographical conditions. Whether this geographical range also encompasses a wide range of environmental conditions is an interesting question as it raises the point as to whether broadly adapted varieties with desirable traits can or should be produced. Experience with other crops suggests that the first round of improved materials may be broadly adapted, but later materials will be more specifically adapted. Certainly most of the currently available information indicates strong genotype by environment interactions, suggesting the future trend should be for improved varieties for particular conditions and possibly end uses.

Agronomic Considerations

Large improvements in yield will not be obtained solely by changing the clones grown but will also require concomitant modifications in agronomic practices. Farm

surveys have shown that yields may be reduced because of diseases and pests, poor quality of planting material, mixed cropping, poor agronomic practices, and low soil fertility.

The cassava crop is vegetatively reproduced using stem cuttings. The large size of these stem cuttings, when compared to seed of most crops, makes cassava planting material relatively robust in tolerating difficult conditions after planting. In addition, the reserves in the planting material provide resilience when it is planted in infertile soils. At the same time vegetative propagation has its drawbacks. Diseases may build up in vegetative tissue and there is no block on vertical disease transmission as often occurs with sexual seed. Furthermore, the vegetative material is not easily handled and stored, and farmers do not normally pay special attention to the production of high quality planting material. Nevertheless, in order to obtain good yields of cassava, particularly under adverse conditions, good quality planting material is a key factor for success.

Once a plantation is established there are many pathogens and insect pests that may attack it and cause severe yield losses. Frequently, a farmer's first reaction to an insect attack on cassava is to apply a potent insecticide. Biological control is often very effective for pests of cassava, particularly in the case of introduced pests. Programs in Africa to control mealybugs and spidermites have been remarkably successful. The search for predators and parasites, under similar conditions in the center of origin to those found in the area where the pest was introduced and thrived, was undoubtedly an important feature of the successful implementation of these programs (Bellotti 2002). As introduced pests are discovered in Asia, the experience in Africa of liberating natural enemies from the neo-tropics is likely to be highly effective.

Sometimes very simple control methods can be effective. For example, root rots, which are common in high rainfall areas, can be greatly reduced by crop rotation and by planting on ridges or mounds, as is traditional in Africa, India, and northeastern Brazil. Other examples could be given, but the few shown here illustrate that when host-plant resistance is not available, diseases and pests can often be controlled without resorting to chemical products. Disease and pest incidence is usually reduced when cassava is intercropped. Cassava yields per hectare in mixed cropping are normally less than when cassava is the sole crop. Yield reduction is even greater when the cropping association is with vigorous, long-season crops. The slow early establishment of cassava makes it possible to intercrop cassava with crops that have a short growth cycle, such as beans and cowpeas, with minimal competition and yield loss. It is more efficient to grow cassava intercropped with such legumes than to grow the root crop and the legumes separately in monoculture (Leihner, 1983.). Hence, total food production per hectare is often enhanced. It is for this reason that much of the world's cassava is grown intercropped. In traditional shifting culture cassava is generally grown with other crops. Nevertheless, cassava is increasingly being grown as a monocrop, and this trend is likely to continue.

Cassava often becomes more important toward the end of the cropping cycle because of its ability to grow on depleted soils. This, however, has led to two misconceptions: first that cassava depletes the soil, and second that cassava does not require fertilization. Depleted soils, that will not support other crops, will often still support

an economic yield of cassava, but to do this they will become further depleted. Hence cassava gains the reputation of a crop that depletes the soil. In fact, nutrient extraction per ton of dry matter harvested is no greater for cassava than for other crops and, with the exception of potassium, cassava actually depletes the soil less than most other crops when nutrient extraction per unit of dry matter produced is considered. Nevertheless, in order to obtain high cassava yields on infertile soils, adequate fertilization is required.

National research expenditure on cassava has been extremely low in comparison with other starchy staples (Cock, 1982). However, over the past thirty years several strong national programs have been established for cassava research, and an international network of cassava researchers has also been established (Thro *et al.*, 1998). Nevertheless, research expenditure on cassava continues to be low.

In the absence of a high research expenditure on cassava much of the development will be done by the farmers themselves. Farmers are always experimenting and trying new things. They are willing to actively participate in research to improve their practices. The much vaunted participatory research builds on the farmers wish to form part of the research continuum. Unfortunately, as currently practiced, much of the participatory research remains extremely location specific and the results cannot be easily transferred to other sites. Furthermore, the results of participatory research are often suspect due to the lack of rigor in their analysis, normally resulting from great difficulties in separating cause and effect of multiple variables with a limited sample number. I suggest that if farmers' practices, with all the variation they encompass, and the conditions under which they work were to be carefully characterized, field by field, then it should be possible to deduce optimal practices for specific conditions (see for example Cock *et al*., in press). This idea would have been utopic twenty five years ago but now with modern information technology it is possible to rapidly characterize the weather and the climate of almost any site in the world and to collect information on soils and crop management, store the data and make valid rigorous analysis of the information compiled in mega-databases.

Repeatedly, cassava farmers have responded to high and stable prices by increasing their production, often through dramatic increases in productivity. They appear to take more care of their crop when they know that they are going to obtain a good price; in the absence of a secure and reasonable price they often leave their crop as a reserve, investing the minimum of their time and effort in an uncertain venture. ***This suggests that solely providing a stable and reasonable price is one of the most effective ways of improving agronomy and crop management.***

Present and Future Potential Demand for Cassava

Cassava's main role in the world economy has been as a basic energy source in the form of starch. Its role is determined by the particular characteristics of the crop. Starch in the form of cassava roots can be produced with relatively low inputs under conditions considered sub-optimal or marginal for other crops; a direct consequence is that cassava is potentially a low cost source of starch. The cassava roots are bulky and highly perishable. In this they resemble such crops as sugarcane and oil palm, which are both almost exclusively grown near to processing plants with production and processing inextricably

linked. This close linkage has given rise to an axiom in the case of sugarcane that goes “Cane without a mill is a weed; a mill without cane is scrap iron”. Thus, we have a product that is highly perishable once harvested and unless processed is extremely bulky. In addition, fresh cassava roots contain the precursors of cyanide, which is released when the roots are macerated. The cyanide, if not reduced to acceptable levels, is toxic to humans and animals. The demand for cassava products is markedly influenced by these characteristics of the crop. It is used as a human food, as a component of livestock feed, as a source of starch and starch derivatives for food and industrial use, and as a primary input for the production of ethanol. Each of these uses is described in turn.

In general, as countries develop and incomes rise, per capita consumption of starch (in many forms) first of all increases, as people eat more. In Africa cassava production is increasingly seen as a major means of providing the supply to meet this initial surge in demand. When basic food needs are satisfied the curve plateaus, only to take off more steeply as incomes increase above a critical point. The increase after the plateau is based on two principle demands: firstly the appetite for more meat, dairy and poultry products after a certain income level (Monke, 2000), and secondly, the use for starch and starch derivatives in a whole series of industrial products. In the developing world an increasing number of countries or regions, particularly in Asia and the New World, are now at the stage of development where this second surge in demand for starch products takes off. Cassava is well placed to satisfy this demand.

In general, prices for starchy crop products are high on a world basis at the moment. Current projections suggest that these high prices are likely to be sustained. As cassava products compete directly with many of these other starchy products on price, the future prices for cassava-based products in general looks good.

1. Cassava as Human Food

In broad terms cassava is consumed as a food directly as fresh cassava, or as moist cassava products based on fresh cassava, or as a series of dry cassava products. With the current increases in food prices, particularly starchy cereal grains, there is an opportunity for cassava to gain importance as a food. It is likely that the opportunities will be greatest in those areas that currently import cereal grains, with emphasis on Africa where cassava is well accepted as a food crop. This view is supported by the experiences in Nigeria: when the government repealed policies that favored low cereal prices, cassava consumption increased dramatically.

Fresh cassava

Probably about half the cassava consumed as food is in the form of fresh cassava (Lynam, 2008). Fresh consumption is much greater in rural than in urban areas. The low level of urban consumption is a reflection of the high perishability of fresh cassava and the high marketing margins that result in high urban consumer cost. If the urban price of fresh cassava could be lowered, the urban consumption might increase. This possibility is supported by the fact that in lower income strata the income elasticity of demand for fresh cassava is high in several countries. Costs can be reduced in part by improved production technology aimed at lowering the "farm gate" price of cassava, and also by improved

storage techniques that reduce both the risks of transporting and of bulk purchasing and, thus, reduce marketing margins. Cassava is a difficult crop to handle in the post-harvest period. Roots start to deteriorate one to seven days after they are harvested. Initial physiological deterioration is later compounded by microbial action. The post-harvest deterioration of cassava can be minimized by various practices including waxing, treatment with fungicides, and storage in humid conditions. Some of these techniques have been adopted on a small scale. It is likely that there will continue to be a demand for whole fresh roots sold directly in local and urban markets, either as simple fresh roots or after simple processing to increase their shelf life.

However, in urban markets and for off-farm consumption there is demand for fresh cassava that has been through a simple process immediately after harvest. In Latin America cassava is increasingly being washed and peeled and then frozen, or grated and made into croquettes before freezing for sale. These products are not well-known in the Asian context: it would seem possible that with active marketing some of these products could carve out a market niche. With the rapid spread of the supermarkets in all developing countries (Reardon *et al.,* 2003) the consumption of fresh frozen cassava products is likely to increase. In Africa many cassava products are made after fermenting the roots and then packing them in a moist form for sale and later consumption.

Dried cassava

At low-income levels demand for fresh cassava is strong, but as income rises, demand flattens off. Dried cassava consumption for the lowest income strata tends to increase with increased income to a point, after which it declines. With the current high prices of cereal grains it is likely that those people in the lower income groups who consume the greatest amounts of cassava flour will consume more, and that those in slightly higher income groups that did not previously consume cassava will purchase cassava as a substitute for higher priced grain products.

As countries develop and incomes rise it is likely that, in the short-term, consumption of simple dried cassava products will increase slightly, but that in the long run the consumption level will decline. At the same time, demand for bakery products is increasing rapidly. Few lowland, tropical, developing countries can meet the present demand for flour for bakery products from their own production, and increasing demand generally leads to ever increasing wheat imports. In order to satisfy urban demand for affordable bakery products, many national governments and aid agencies in the past heavily subsidized locally produced and imported wheat. These subsidies make it difficult for wheat flour substitutes to compete and, hence, may prevent the development of local alternatives. It is technically feasible to substitute wheat flour with 10 to 20 percent cassava flour, yet this rarely occurs. This is partly because of the wheat subsidy, partly because of the lack of supplies of dried cassava flour, and partly because the products with wheat flour substitutes are never quite as good as the pure wheat flour product. On the other hand, there are a series of local bakery products made from cassava starch or fermented cassava starch (eg: pao de queijo in Brazil and pan de bono in Colombia) that are not considered to be inferior goods; in fact, they are highly prized. I would suggest that there is a greater opportunity to develop products that build on the particular traits of cassava flours and

starches rather than substitution into wheat products. Krupuk, a highly prized snack in Indonesia, is a good example of how this approach can be successful. Similarly, cassava based noodles are becoming popular in parts of SE Asia.

2. Cassava as Animal Feed

As countries develop above a certain level, their demand for animal products increases markedly. The demand is largely met from products intensively produced using balanced feed rations, either as the complete diet or as an essential supplement, or with on-farm feeding. The surge in demand is already occurring in Asia and Latin America[2]. In southern Brazil and Vietnam pig production has been supported successfully by on-farm feeding of cassava. This can be done directly with fresh cassava, silage or dried cassava products. The experience in Europe with Thai products has confirmed the technical feasibility of producing dried cassava products and using them in balanced diets. With the current high prices of all basic energy crops the demand for cassava as animal feed is likely to increase.

Foreseeing the demand for cassava as an animal feed, at various times the idea of producing a high protein cassava has emerged. This concept is superficially attractive; however, it needs to be treated with caution. A large proportion of the nitrogen in the cassava roots is non-protein nitrogen, hence it is easy for people to be misled believing that high nitrogen cassava is also high protein cassava and will provide useful protein in animal feed. In addition, as we have already noted, cassava as a crop excels in low fertility conditions, including those where nitrogen is in short supply. *A priori* it would seem likely that high protein cassava would not thrive and do as well as normal cassava under these conditions, or if it did and the roots were harvested and removed from the system, soils would be severely depleted. Thus, while it is not possible to totally discard the idea of high protein cassava as a viable alternative, it would be advisable to explore the possible agronomic disadvantages of high root protein and confirm that it provides a better quality feed before embarking on an expensive improvement program.

3. Cassava Starch

The rapid development and increased incomes in much of the developing world will be accompanied by an increased demand for starch for food and industrial use. Once again, the high prices of many other sources of starch suggest a major opportunity for marketing cassava starch. In addition, the recent discovery of low- or zero-amylose starch in cassava genotypes produced by selfing suggests that specialized starches can be produced. Under these conditions the potential for cassava starch seems excellent.

4. Cassava as a Renewable Energy Source

Out of interest, the precise statement first published in Science in1982 (Cock 1982) is repeated here. *"Dwindling fossil fuel supplies have resulted in renewed interest in*

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[2] In large parts of Latin America there is a long history of eating animal products with beef as part of the culture. This was largely produced on the extensive grasslands of the region. More recently, demand for pig and poultry products has surged.

alternative energy from biomass. Cassava is frequently mentioned as a potential biomass crop because of its ability to produce high yields of carbohydrates. These carbohydrates can be used to produce ethanol. Brazil has vast areas of acid, infertile soils that are currently underutilized. It is in these areas that very small amounts of cassava are grown as a substrate for grain alcohol production. Locally grown tree crops are used to fire the boilers for the anhydrous ethanol production. With this system net energy ratios (NER) are positive. If, however, fossil fuels are used in the distillation, the NER is barely greater than one. This suggests that where liquid fuel is in short supply, and where sources of non-liquid energy such as coal are available, cassava may indeed have a role to play. In other areas the NER could be improved by using cassava stalks as an energy source in a manner similar to the use of sugarcane bagasse, but this has not yet been achieved even on an experimental basis. With currently available technology, 70 percent of the energy used in alcohol production from cassava is used in the industrial process, mainly in the separation of alcohol from water. Until this requirement can be reduced, the benefits of using cassava for alcohol production are questionable. However, more energy-efficient separation methods are being developed. These could radically alter the potential use of cassava in energy production."

Although there have been minor improvements in separation methods, major breakthroughs have not been forthcoming. The question then becomes as to whether all the efforts and the enthusiasm for cassava ethanol as a renewable energy source are justified. As described at the beginning of this article, cassava is not a particularly efficient producer of total biomass; rather, it is an extremely efficient producer of carbohydrate in the form of starch. Starch (or the sugars into which it is hydrolyzed) are not by any means an ideal raw material for producing liquid fuels. The starch has to be converted into alcohol in solution, and then the alcohol has to be separated from the water. This latter process is extremely energy consuming. Sugarcane obviates this problem as the crop is a high biomass producer and the bagasse, that is produced as a co-product, provides more than enough energy to power the extraction processes and separate the alcohol from the water. Returning to cassava, in a recent paper Du Dai *et al.*(2006) clearly showed that even with the latest technology, for every joule of fossil fuel input the output of energy (net energy ratio, NER) from cassava ethanol was 1.27 joules, a minimal improvement over the value of 1.21 joules reported in my original article in 1982. Under these conditions, cassava ethanol is not an attractive option when compared with the values obtained from sugarcane alcohol or many of the biodiesel alternatives. However, Du Dai *et al.*(2006) clearly indicate the interest of China in cassava ethanol: *How to convert coal into a liquid fuel that vehicles can use is a problem that China has faced for decades. Through fuel ethanol production, the abundant domestic coal, combined with other renewable energies, can be converted into premium liquid fuel. Thus, it provides a gasoline substitute for urban transportation and reduces oil imports.* Thus, for ethanol as an energy source the main option would currently appear to be as a way of converting coal or other energy sources into a liquid fuel, with a small energy gain in the process, rather than as an option for renewable energy. At the same time it should be noted that advances in technology to separate the water from the ethanol could radically change the situation if they were to become available.

Final Commentary

Expansion of cassava production can increase the incomes and livelihoods of producers, whilst at the same time providing society at large with a feedstock for its increasing demand for starch products. Increased production will be stimulated by generally higher prices of all starch products over the coming years than those prevalent up to about 2005. Production technologies are being developed to produce cassava as a source of starch in the poorer agricultural lands where cassava has a comparative advantage. The farmers, processors and traders involved in the production of increasingly sophisticated cassava-based products will only be successful if the services and infrastructure required for their activities are in place. Furthermore, cassava producers will not be able to successfully expand production if, as occurred frequently in the past, policies and subsidies favor other competing products.

Within these restrictions it can safely be said that cassava is a basic energy source whose time has come in a world of scarce energy and greater environmental concern.

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CHAPTER 3

CASSAVA GROWTH AND DEVELOPMENT

James H. Cock[1]

INTRODUCTION

This paper is not envisaged as a review of the physiology of cassava. For recent comprehensible and comprehensive reviews on this topic the reader is encouraged to read El Sharkawy (2003, 2006) and Alves (2002). The objective of this chapter is to provide cassava agronomists, soil scientists, plant protection experts, breeders and others interested in cassava production with an understanding of how the crop functions and responds to varying circumstances. Hopefully, the insight gained can then be used to better manage and improve the crop.

Cassava tends to be grown on the poorer agricultural lands, without irrigation and with limited application of purchased inputs. It is naturally well adapted to these conditions (Jones, 1959; Cock and Howeler, 1979; Cock, 1982; 1985). Nevertheless, the tremendous variation in conditions to which it is subjected means that cassava production technology needs to be adapted to the varying natural conditions, rather than using costly modifications of the environment to suit a particular production system (Kawano and Cock, 2005). An understanding of the manner in which the crop responds to varying environmental conditions is an essential component of designing improved low-input technologies, well adapted to the particular conditions where individual farmers grow their crops.

Origin and Adaptation

Cassava (*Manihot esculenta* Crantz) is a perennial shrub grown principally for its starchy roots which are used as food, animal feed and as a source of starch. In some areas the leaves are used as a food or as a protein source for animal feed. The center of origin of the crop is in the neo-tropics (South America) with a major center of diversity of *Manihot* spp. in Brazil, and a secondary center in Meso-America (Central America). Renvoise (1973) suggested that the sweet cassava varieties may have been domesticated in Meso-America and the bitter types in northern South America, whilst observing that there is no sharp demarcation between the sweet and bitter types. On the other hand, Gibbons (1990) suggested that low cyanogen cultivars were first domesticated in the Amazonian jungle. Until recently it was assumed that wild populations of *Manihot esculenta* did not exist. However, Allem *et al.* (2001) suggests that *M. esculenta* Crantz ssp. *flabellifolia* (Pohl) Ciferri is the likely ancestor of cassava, and that it is currently found in the wild and able to cross freely with cassava. In fact, it is synonymous with *M. saxicola*, which Nichols (1947) noted crossed readily with *M. esculenta* in Tanzania and is probably not a separate species (Allem *et al.*, 2001; Allem, 2002; Nichols, 1947).

The cassava crop is essentially grown between 30^0 S and 30^0 N latitude. Near the equator it can be found at altitudes up to about 2000 m. As the crop moves further north or south of the equator the maximum altitude at which it grows and produces will decrease.

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The crop is generally not found in areas where the mean average temperature is less than about 20^{o}C, although in areas near the equator where seasonal temperature fluctuations are small it can be found growing in areas with a mean temperature as low as 17^{o}C (Cock, 1982; 1985).

Most cassava is grown in areas where average rainfall is over 1000 mm/year, although it is also found in areas with as little as 750 mm/year average rainfall and with some years with as little as 600 mm/year distributed over as little as five months. The crop can be found in areas with rainfall as high as 3000 mm/year, but it will not stand poor drainage. On heavy soils, one day of flooding can kill the crop.

Cassava is well adapted to low fertility soils that predominate in large areas of the tropics. It is frequently cultivated on the highly weathered and leached Oxisols, Ultisols and Alfisols, with smaller areas found on Inceptisols (particularly in India) and Entisols. Cassava is extremely tolerant of low soil pH and high levels of aluminum saturation that often accompany them. It can be found producing moderate yields where many crops simply fail due to the low pH and high levels of aluminum (Cock, 1982; 1985; Cock and Howeler, 1979).

Morphology, Growth and Development

The cassava plant has a relatively simple structure in commercial production. The basic constituents of the plant are (i) nodal units that consist of a leaf blade, petiole and internode and (ii) thickened roots that form at the base of the original cutting or the axillary buds on the planting piece. Flowers may be produced but they are not important in commercial production. The stem is formed from a number of nodal units. The leaves that form part of the stems produce carbohydrate. This carbohydrate is then used partially to produce and maintain nodal units and partially to support root growth. The delicate balance between top growth and root growth is the key to understanding the growth and development of cassava (Cock, 1976). Excessive top growth leaves little substrate for root growth, whereas limited top growth reduces the capacity of the plant to intercept solar radiation and use that energy to produce carbohydrates to fill the roots.

Clonal propagation

Cassava producers use vegetative stem cuttings to clonally propagate cassava. The plant can also be propagated by seed, but every seedling is genetically distinct and hence seed propagation does not lead to a uniform crop. On the other hand, genetic improvement programs make crosses and use the sexual seed produced to develop new varieties.

Hardwood cuttings are taken from cassava plants that have developed sufficiently to produce woody stems. Cuttings are normally taken from plants that are more than six months old. Long (1 m or more) stem pieces can be stored for up to six months in the shade, or in special underground structures in areas where frost is a problem. The stored stem pieces frequently sprout at the tips: this sprouted material is normally discarded. It is advisable to treat the stored cuttings with fungicides to conserve them (Leihner, 1984). Cuttings stored in this manner produce yields comparable to fresh cuttings (Leihner, 2002). In commercial practice, cuttings are normally 10-20 cm long; they may be planted horizontally and covered with a few centimeters of soil, or vertically or inclined with a third or less of the cutting protruding from the soil. There has been much research on the effects of orientation of the cuttings in the soil on subsequent growth, development and yield with no consistent effects (Toro and Atlee, 1980; Leihner, 2002). Sprouting of the

cuttings is extremely temperature sensitive. At 16^{o}C sprouting was delayed in all varieties, and the percentage of cuttings that sprout in some varieties was only 20% (Cock and Rosas, unpublished data). Sprouting is most rapid at 28.5-30^{o}C with no sprouting above approximately 38^{o}C, and decreasing markedly below 17^{o}C (Keating and Evenson, 1979).

At optimal temperatures of about 30^{o}C, after 5-8 days adventitious roots emerge from the base of the axillary buds and callus forms on the basal end of the original stem cutting. Simultaneously, the axillary buds expand and after ten days leaves begin to appear. Once the axillary buds begin to expand there is a strong apical dominance effect, which suppresses the development of all but one or two and occasionally three buds per cutting (Wholey and Cock, 1974). The apical dominance appears to be affected by the orientation of the cuttings with horizontal cuttings producing more shoots per cutting.

The initial growth and development of the plant depends on the reserves in the original stem cutting. Thin cuttings from the upper part of the plant tend to have low reserves of carbohydrates and develop slowly. Mineral reserves are also extremely important (Cock, 1984) and depend on the fertilizer treatment provided to the mother plant (**Table 1**). The photosynthetic rate of plants obtained from mother plants that have not been fertilized is similar to that of fertilized mother plants (Cayon *et al.,* 1997). Nevertheless, the status of the nutrient reserves is important for the initial establishment of the crop, and in turn this early establishment is important in determining final yield (Molina and El Sharkawy, 1995). Good quality planting material is essential for obtaining good yields.

Table 1. Effects of fertilization of mother plants on the yield of daughter plants.

Fertilizer treatment N-P-K (kg/ha).	Fresh root yield (t/ha)
0-0-0	19.1
100-87-125	26.2

***Source**: CIAT, 1981.*

Growth and development under field conditions

The variation in growth habit of cassava varieties is large. Furthermore, old landrace varieties exist that are well adapted to specific conditions. Thus, for example, farmers have selected varieties that only perform well in the highland tropics (1500-2000 masl) near the equator. Given the large number of genotypes of cassava grown commercially, and the diverse ecosystems in which the crop is grown, coupled with the interaction between genotypes and the environment, it is difficult to provide a generalized morphological description of cassava (Alves, 2002). In the following sections the overall growth and development of cassava is described, referring to both general characteristics and also specific adaptation to particular conditions.

Apical dominance

The shoots show marked apical dominance and new leaves are produced in sequence along each shoot. Lateral shoots occasionally develop from axillary buds on the lower stem to produce lateral branches (see **Figure 1**). The apical meristem may become damaged by disease or insect attacks, or when young leaves are harvested as a vegetable. When this occurs the lateral buds immediately below the damaged meristem expand, but due to apical dominance normally only one lateral shoot develops. When vigorous

plantations of cassava lodge with many horizontal stems, the apical dominance is reduced and several axillary buds form new shoots.

Nodal units

The shoot of the plant in the vegetative phase consists of nodal units comprised of an internode with an axillary bud and a leaf. Each node forms a palmate leaf with the number of lobes almost always being odd, but with numbers varying with the age of the plant, the nutritional status and general growth conditions. I have observed leaves with up to 13 lobes, and furthermore, a decrease in the number of lobes immediately before forking (see below). The leaf blade is supported by a petiole, which is botanically part of the leaf, and at the base of which is an axillary bud. The plant develops by producing new nodal units, generally from the apical meristem. Nevertheless, as we observed above, occasionally lateral branches form. A further type of branching, generally called forking, occurs at intervals. The apical meristem becomes reproductive, and even if a flower structure does not develop, two, three or four axillary buds develop immediately below the reproductive apex and form similar sized branches (see **Figure 1**).

Branching

Little is known about the control of forking in cassava, and consequently little about flowering. Photoperiod affects the formation of reproductive apices in cassava with long days substantially increasing the amount of forking with the first fork occurring earlier (Cock and Rosas, unpublished data; Veltkamp, 1985; Keating *et al.*, 1982a; Conceicao, 1979). Keating *et al.* (1982a) also indicated that cooler night temperatures promoted flowering and hence branching. Some clones will fork early and continue branching whilst a small number have never been known to branch.

Obviously these non-branching types cannot be included in conventional breeding programs as they do not produce flowers. Under constant environmental conditions the interval between successive branches tends to be constant when the number of branches at each fork is small, but increases when branch number is large (Tan and Cock, 1979a). When growth is restricted due to water or nutrient stress, fewer forks are produced and intervals between forks increase (Connor and Cock, 1981). The first forking is delayed at lower temperatures (20^{o}C) and higher temperatures 28^{o}C (Irikura *et al.*, 1979).

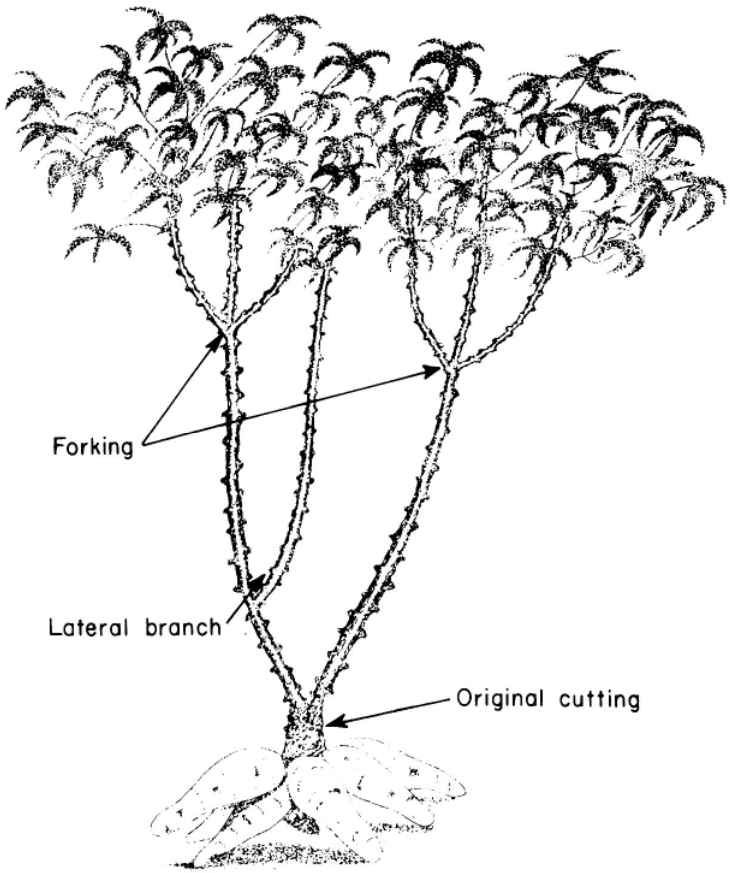

Figure 1. Components of the cassava plant.

Flowering and breeding

Flowering is essential for breeders. Often the first forking does not produce flowers, although in the V formed by the fork the vestiges of the initiated florescence can be seen. Cassava is monoecious, producing both male and female flowers on the same inflorescence. The female flowers are larger, but fewer in number than the male flowers which are found at the tip of the florescence. The female flowers open 1-2 weeks before the male flowers in the same inflorescence (Alves, 2002). In heavier branching types, male and female flowers may open at the same time at different branching points. Under natural conditions cassava is cross pollinated by insects but considerable selfing may also occur.

The fruit which matures two to three months after pollination is a trilocular capsule containing three seeds. On maturing the fruit dehisces and ejects the seeds, which each weigh 95-135 g (Alves, 2002).

Some breeding programs have been based on collecting seeds from open pollination in germplasm banks. This system profuse early flowering types. As flowering is directly related to branching the result may be a large number of heavy branching progeny

which is not desirable. This viewpoint is supported by the work of Pellet *et al.* (1993a) in which root yield was negatively related to the weight of reproductive organs collected.

Rate of formation and growth of nodal units

The rate of appearance of new nodal units from a single apex is greatest when the plant is young (**Figure 2**) reaching about one leaf per day per active apex, declining to one per week in older plants with little difference between varieties. The rate of formation of new leaves declines as temperature decreases (Irikura *et al.,* 1979) and is reduced under water stress (Connor and Cock, 1981; Cock *et al.,* 1985).

The internodes themselves serve a supporting role to maintain the leaf canopy and to translocate photosynthate to the roots. The individual internodes and the internodes on the original planting piece continue to grow throughout the life of the crop (Tan and Cock, 1979b). The original cutting also stores starch and at one time a starch factory in Australia harvested the original cutting with the roots, milled it and extracted significant quantities of starch from it (Noel Harris, pers. comm.).

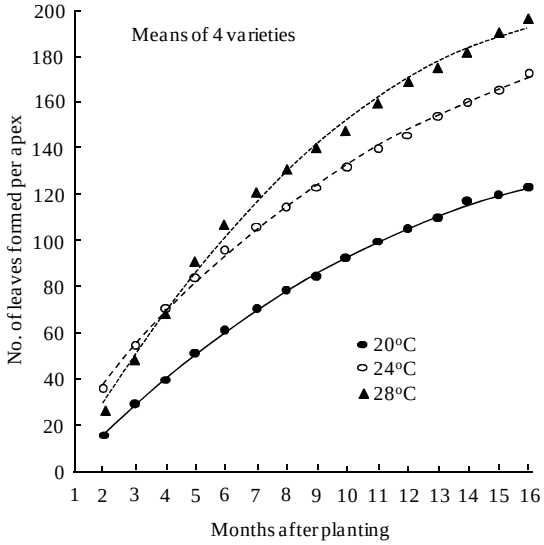

Figure 2. Cumulative number of leaves formed per apex at various temperatures.
Source: *Irikura et al., 1979.*

Leaves

A new cassava leaf is produced at each nodal unit, except when the apex becomes reproductive. The first fully expanded leaves are small but they rapidly increase in size reaching their maximum size four to six months after planting depending on the temperature (Irikura *et al.,* 1979). Keating *et al.* (1982a) found that under certain conditions maximum leaf size occurred earlier. Leaf size in one variety was increased under long days (Keating *et al.,* 1982a). The maximum life of a leaf after appearance is about 200 days under cool temperatures (Irikura *et al.,* 1979). Under normal conditions leaf life is of the order of 60-180 days in the first four months of growth increasing to about 120 days at later growth stages. There are large varietal differences in leaf longevity (Tan and Cock, 1979a; Cock *et al.,* 1979; Lenis *et al.,* 2006). Leaves fall when an abscission layer forms at the base of the leaf. When leaves are subjected to heavy shading they fall within about ten

days (Rosas *et al.*, 1976; Cock *et al.*, 1979). This appears to be a hormonally controlled response as the effects of shading can be nullified by applications of hormones (Rosas *et al.*, 1976). The shading effect is readily seen when one approaches a dense stand of cassava about six months old; leaves on the outer side of the plots that are well illuminated reach almost to the ground, but inside the canopy only the upper leaves are still attached to the stem. Water stress reduces the production of new leaves and may increase the longevity of leaves (Connor and Cock, 1981).

Roots

The original hardwood cutting produces fibrous roots from the axillary buds and also the callus that forms at the base of the cutting. From as early as 25 days after planting starch is deposited in the fibrous roots. After about two months secondary thickening of some of the fibrous roots is visible and starch is deposited in the parenchyma (Cock, 1984). The number of thickened roots is determined early in the growth cycle, normally in the first three months (Wholey and Cock, 1974). The number of thickened roots varies with conditions and the variety with up to 19 thickened roots reported by Wholey and Cock (1974). Cock *et al.* (1979) and Tan and Cock (1979a, 1979b) suggested that top growth has preference over root growth. This suggests that roots start to thicken when the tops produce excess carbohydrates to their needs.

The fibrous feeder roots of cassava were described as being rather sparse by Connor *et al.* (1981), reaching a maximum level of 1 km/m^2 and depths of at least 2.6 m. However, Aresta and Fukai (1984) found much greater root densities of 20 km/m^2 or more, which is in line with levels encountered in drought tolerant crops such as sorghum. In later trials, El Sharkawy and Cock (1987 b) also found higher densities than those reported by Connor *et al.*, (1981) but with the highest values about 3 km/m^2 . The fibrous feeder roots reach to a depth of 2 m or more and are capable of extracting water at this depth.

Photosynthesis

Most crop plants possess either the C3 or C4 photosynthetic cycle. The C4 plants tend not to light saturate, have low photo-respiration, high photosynthetic rates on a per unit leaf area basis and hence are also nitrogen and water use efficient. Cassava has normally been considered to be a typical C3 plant (for example Mahon *et al.*, 1977a; 1977b; Angelov *et al.*, 1993; Aslam *et al.*, 1977; Calatayud *et al.*, 2000b; Edwards *et al.*, 1990; Alves, 2002). The photosynthetic rates reported by Tan (unpublished data) and El Sharkawy (2006) for cassava in field grown plants (40 μmol $CO_2/m^2/s$) are high for a C3 plant. Furthermore, wild species of *Manihot* have photosynthetic rates as high as 50 μmol $CO_2/m^2/s$ (El Sharkawy, 2006). Work at CIAT suggested that cassava might be a C3-C4 intermediate (Cock *et al.*, 1987; El Sharkawy and Cock, 1987a). El Sharkawy (2003, 2006) presents much evidence and argues that cassava and some wild *Manihot* spp represent an intermediate photosynthesis evolving from C3 to the C4 species (El Sharkawy *et al.*, 2008).

Connor and Palta (1981) found that cassava stomata closed in well-watered and stressed plants in the field at midday. Cassava stomata are extremely sensitive to the Vapor Pressure Deficit (VPD) between the leaf and the air (El Sharkawy *et al.*, 1984; 1985; Palta, 1984; Cock *et al.*, 1985; El Sharkawy and Cock, 1984; 1986; El Sharkawy *et al.*, 1984; 1985) (**Figure 3**). Even well-watered plants in an environment with a large VPD close their stomata and show reduced photosynthesis and growth in the field (Cock *et al.*, 1985). The stomatal response is so strong that the water potential (a measure of the level of stress) in

the leaves of unwatered plants may be similar to that of well-watered plants in the field. This high degree of stomatal sensitivity is a major factor in making cassava so tolerant of drought. The stomatal sensitivity is greater in plants with less soil moisture. The partial stomatal closure reduces both overall growth and root yield. Nevertheless, under conditions of highly available soil water, as for example in the irrigated cassava fields in Tamil Nadu, the highly sensitive nature of stomata may be a disadvantage. The varieties grown in Tamil Nadu are specialized varieties and differ from those grown under rainfed conditions in the neighboring state of Kerala.

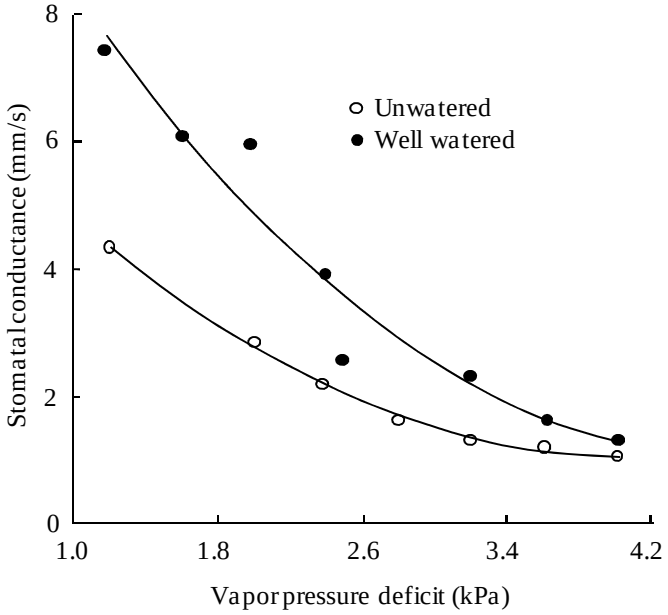

Figure 3. Response of stomata to leaf to air vapor pressure deficit in well-watered and unwatered cassava plants.
Source: *El-Sharkawy et al., 1984.*

Photosynthesis of cassava leaves in the field declines with leaf age (Cock *et al.*, 1985) This decline is probably large due to the low light environment of older leaves. If leaves are well illuminated they can maintain photosynthetic rates at a high level during substantial periods (**Figure 4.**). Varieties that maintained their leaves for a longer time produced greater yields than those with shorter leaf retention, indicating that older leaves can photosynthesize actively and that longevity of leaves is a desirable trait.

The photosynthesis of leaves of plants grown at lower temperatures (18^{o}C) tends to be lower than those of plants grown at 24°C or above (El Sharkawy, 2006). Photosynthetic rate generally has a broad plateau over the range of 25-35°C leaf temperature, declining to zero at 50^{o}C and is greatly reduced at 15^{o}C (El Sharkawy and Cock, 1990).

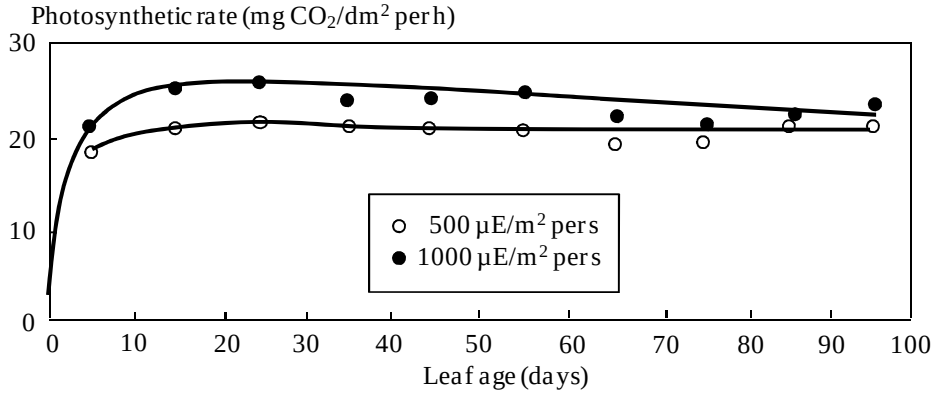

Figure 4. Photosynthetic rate of leaves of cassava, MCol 72, at two light levels.

A whole series of trials have shown a relation between photosynthetic rate of individual leaves and the root yield of cassava under stressed and unstressed conditions. Recently, El Sharkawy *et al*. (2008) found that photosynthetic rate measured in preliminary yield trials was correlated with root yield in subsequent independent yield trials. This suggests that this is a genetically controlled trait. It is recommended that breeders should select for increased photosynthetic rate, combining this trait with other desirable characteristics (El Sharkawy, 2006). Increased yield associated with increased photosynthetic rate, as expected, increases the nitrogen use efficiency (El Sharkawy *et al.*, 2008), and also, presumably, water use efficiency. Furthermore, recently it has been shown that activity of PEP carboxylase, an enzyme associated with C-4 photosynthesis, is correlated with photosynthetic rate and yield. Thus, it might be easier to screen parent materials for crosses for their PEP carboxylase activity in breeding programs.

Leaf Area Index, light interception and crop growth rate

The total biomass produced by a crop is influenced directly by the efficiency with which the intercepted solar radiation is used by the leaves and the proportion of the solar radiation that is intercepted. The overall total biomass production of a cassava stand is closely related to the total amount of light intercepted (Veltkamp, 1985). Light interception in turn is closely related to Leaf Area Index (LAI), defined as the one sided area of leaf lamina per unit of land area. The development and maintenance of LAI depends directly on the number of plants per unit land area, the number of leaves produced per plant, the size of those leaves and their longevity. The development and maintenance of LAI varies tremendously depending on the values of the different parameters. In general, LAI increases with crop age and then declines towards the end of the crop cycle. However, this pattern varies widely. For example, in semi-arid areas cassava is often grown for a period of 18 months or more. Under these conditions LAI builds up rapidly after planting when the rains initiate, but with a prolonged period of six months or more of drought most of the leaves fall and there is a new flush of leaves with the onset of the next rains. Similarly, in

areas with cool winters the crop cycle is often more than one year and with the cool weather, or light frosts, all leaves fall, followed by a new flush of leaves in the following spring.

Light interception increases with LAI according to the formula $\ln I/I_0 = -k.LAI$ where k is the Extinction Coefficient, I is the light below the LAI and I_0 is the incident light on the canopy. Extinction coefficients in cassava generally fall between the values 0.6-0.88 when full canopy cover has been reached (Fukai *et al.*, 1984; Cock *et al.*, 1979; Veltkamp, 1985). These data indicate that a cassava crop intercepts about 90% of the total solar radiation when it reaches a LAI of 3-4. Cassava rarely maintains LAI of more than 4-5 for a long period, as shading of the lower leaves causes them to abscise (Rosas *et al.*, 1976; Cock *et al.*, 1979). A major exception is that of cassava grown in Australia at higher latitudes with long days and high solar radiation with leaf area indices greater than 6 (Fukai *et al.*, 1984; Keating *et al.*, 1982a; 1982b). Similarly, Cours (1951) in Madagascar at 17°S latitude reported higher LAIs. Irikura *et al.* (1979) also reported that one variety from a highland area maintained an average LAI of 5-6 over an eight month period when moved to lower sites with higher temperature.

Maximum reliable recorded crop growth rates of cassava are of the order of 20 g/ m^2/day (**Figure 5**) (Keating *et al.*, 1982b). The rates are highest at higher LAIs and with higher solar radiation and long days with a direct relation between crop growth rate and solar radiation (Keating *et al.*, 1982b). Under conditions near the equator with day lengths close to 12 hours, 15 g/m^2/day can be obtained with LAIs of 3 or more (Cock *et al.*, 1979). Keating *et al.* (1982b) point out that these crop growth rates are not exceptional, suggesting that the high yielding capacity of cassava is not due to inherently high crop growth rates.

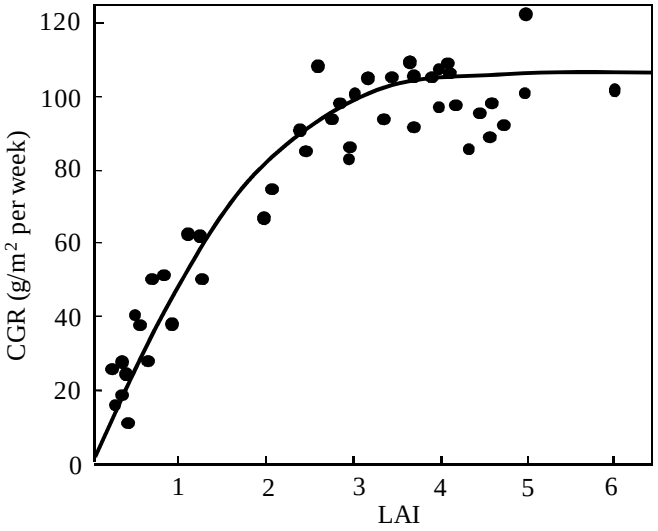

Figure 5. The effect of leaf area index (LAI) on crop growth rate (CGR).
Source: *Cock et al., 1977; 1979 .*

The leaves have several mechanisms to maximize interception of solar radiation, and also to reduce heat load on the leaves and transpiration (El Sharkawy and Cock, 1984; Berg *et al.*, 1986; Calatayud *et al.*, 2000a). The petiole of the cassava leaf is about 10-25

cm long and constitutes 20–30% of leaf weight. The petiole plays a key role in orientating the leaf blade towards the sun during the day, maximizing light interception early in the morning and in late afternoon in a heliotropic response (El Sharkawy and Cock, 1984). Similarly, Tan Swee Lian and James Cock (unpublished field observations) looked at the effects of shading on leaf life and found that the petioles twist and bend so as to avoid shade on the leaf blade. This effectively produces a mosaic of leaves, maximizing the light interception per unit LAI. The effects of these variations on leaf disposition have on light interception have not been studied in depth. This may explain some of the high extinction coefficients (0.88) reported by Veltkamp (1985). Re-examining the work of Veltkamp (1985) it appears that extinction coefficients are greater at LAIs up to about three and then decline; this is probably due to the mosaic effect with changes in leaf angel and disposition being more important at low to intermediate LAIs.

Leaf folding or drooping of plants grown in the field decreased light interception by 50% compared with adjacent horizontally artificially supported leaves, reducing the leaf temperature by 6-7^{o}C and the leaf to air VPD by about 1.3 KPa. The stomatal conductance in the folded leaves was more than double that of nearby horizontal leaves (Berg *et al.*, 1986). The leaf folding mechanism reduces incident radiation on the leaves and prevents damage to the photosynthetic system (Calatayud *et al.*, 2000).

Root dry matter content

The dry matter content of cassava roots ranges from about 25% to up to 40%. Dry matter content is an extremely important characteristic of cassava, particularly if the roots are to be processed. In industrial crops with a high water content the costs of harvesting, transport to a processing factory and the primary processing are all directly proportional to the fresh weight of the product, whereas the value of the product is in the dry weight. Hence, it is more cost effective to produce high dry matter products (Cock *et al.*, 2000).

The dry matter content of cassava roots is a varietal characteristic. Some varieties tend to always produce higher dry matter than others. Nevertheless, the dry matter content is also determined by the growing conditions. Cassava starch factories around the world know that after a dry period, with the flush of new leaves at the onset of the rains, the dry matter content of the roots drops dramatically, but then increases once a new leaf canopy has formed. The drop in dry matter content is probably due to mobilization of starch reserves in the roots to support the flush of new leaves (Lenis *et al.*, 2006).

THE FUNDAMENTAL BASIS OF ROOT YIELD IN CASSAVA

During most of its growth cycle cassava plants simultaneously produce leaves, stems and roots. In this respect cassava differs markedly from cereal and other determinate growth habit crops, which first of all produce the photosynthetic apparatus (and some reserves in certain crops) and then use that photosynthetic apparatus (and reserves) to fill the economically useful plant part. This simultaneous growth of the leaves and the stems that support them, which can be considered as the carbohydrate factory, and the roots, which are like a warehouse to store starch, leads to a delicate balance between maintaining the factory and filling the warehouse.

The distribution of biomass to the shoots, roots and leaves is the subject of considerable discussion. Boerboom (1978) suggested that once a critical plant biomass was reached the proportion of biomass proportioned to the roots and the stems remained constant. This view was supported by the data of Veltkamp (1985) working under relatively

uniform environmental conditions during the growth cycle. This constant proportion theory is not supported by data collected in less uniform conditions. Patterns of dry matter partitioning among plant organs are affected by changes in soil nutrient level, water regime, solar radiation, length of the day (photoperiod) and temperature (Irikura *et al.*, 1979; Connor *et al.*, 1981; Fukai *et al.*, 1984; Veltkamp, 1985). Keating *et al*. (1982c) used a multiple regression to analyze the distribution ratio (DR), which is the proportion of total dry biomass formation over a period of time found in the roots. The DR decreased with increasing LAI and with decreasing temperature. The simple model of Boerboom (1978) has been superseded by the shoot and leaf preference hypothesis. This proposes that the requirements of building and maintaining the leaves and stems are first met, and any excess to those needs is passed to the roots (Cock *et al.*, 1979; Tan *et al.*, 1979a). Keating *et al.*, (1982c) describe this model as assuming "that storage roots receive only that assimilate remaining after the requirement for shoot growth…." and adhere to the idea that LAI can be used to indicate the size of the preferential sink. This hypothesis is supported by much data including, *inter alia*, the increased DR at low nitrogen levels, lower temperatures or in water stressed plants, all of which decrease the top growth (Irikura *et al.*, 1979; Connor *et al.*, 1981; Keating *et al.*, 1982c; Cock and Sharkawy, 1988a; 1988b). On the other hand, the DR decreases at high plant populations (Cock *et al.*, 1977) and with long days (Veltkamp, 1985; Keating *et al.*, 1982c), which both tend to increase the requirements of leaves and stems.

Under non-stress conditions, crop growth rate (that can be used as a proxy for photosynthesis minus respiration) increases with LAI, and reaches a plateau at LAI of 3-4 (Cock *et al.*, 1979). Keating *et al.* (1982b) found the plateau at higher LAIs but observed that they had few data points at those high LAIs. The measured extinction coefficients suggest that 90% of the incoming radiation is intercepted at LAIs of 3.3-3.8, with 95% interception at LAIs of 4.3-5.0; thus, it is most unlikely that crop growth rate will increase substantially above LAI of 4-5. When LAI is neither increasing nor decreasing, a constant amount of photosynthate is required to maintain a given LAI. The relation between crop growth rate, maintenance of LAI and root growth was estimated (Cock, 1980; 1984). On the assumption that the proportion of the crop growth rate needed to maintain a given LAI increases linearly with LAI, the excess carbohydrate left over for root filling increases to an optimum LAI and then decreases (**Figure 6**).

The principles used to develop the model of balance between leaves, stems and roots and the shoot preference model that lead to an optimal LAI can be applied to cassava grown under stressed and unstressed conditions. The same principles apply over a wide range of conditions but the optimum varieties and management practices will vary depending on the conditions.

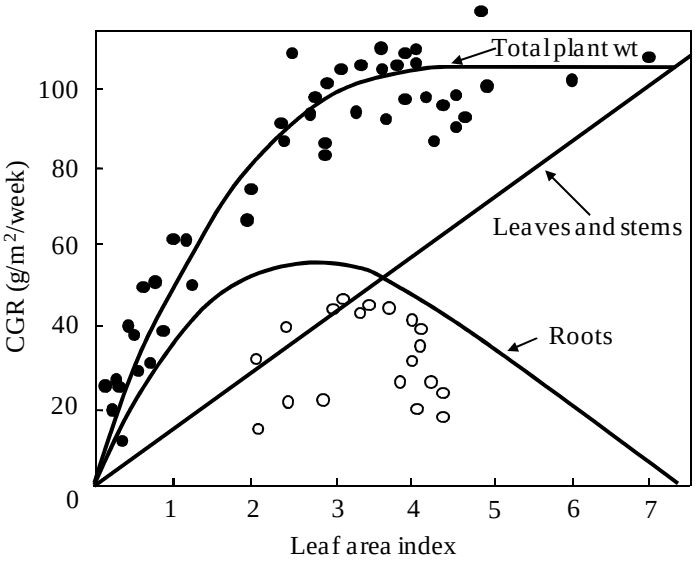

Figure 6. Crop growth rate, stem growth rate and root production over a range of LAI.
Source: *Cock, 1980; 1984.*

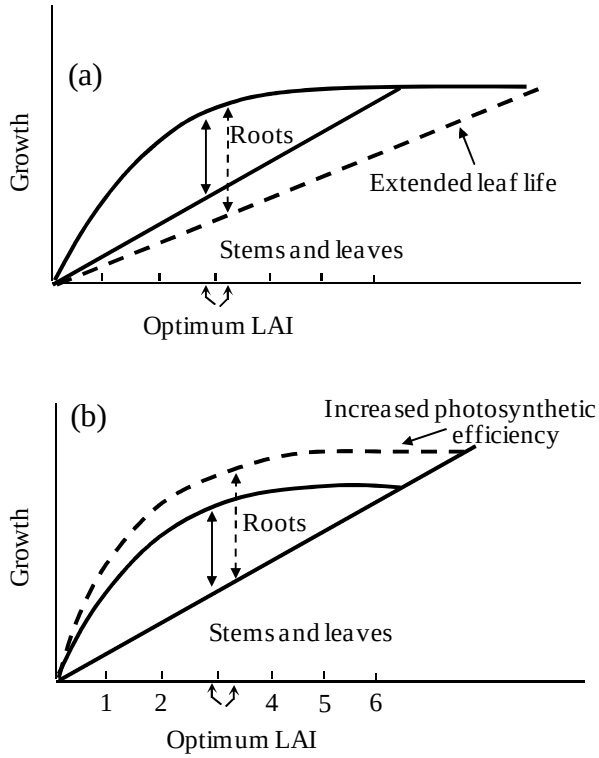

Figure 7. Possible means of increasing root yield of cassava by increasing leaf life (a) and increased photosynthetic efficiency (b).

Production Under Minimal Stress Conditions

At this point we shall look at production under minimal stress conditions, and then from that basis we shall establish how to optimize production under stress conditions. In this context it is noteworthy that large vapor pressure deficits (VPDs) between leaf and air, which are not normally taken into account as a stress factor, are included due to the particular characteristics of the cassava crop.

Under near ideal conditions, Cock *et al.*(1979) suggested that a cassava crop should have the following characteristics: (1) first branching at about 30 weeks in order to maintain leaf production as the rate of leaf formation per apex declines; (2) a leaf longevity of 15-20 weeks, which achieves a balance between excessive dry matter for new leaf production and the practical problems of maintaining leaves active for longer periods in the presence of diseases and pests; (3) a maximum leaf size of 500-600 cm^2; (4) two shoots per cutting at a planting density of 10,000 plants per hectare; and (5) at least nine thickened roots per plant. These characteristics lead to a crop which maintains a LAI near the optimum of 3-4 during a large part of the growth cycle. Implicit in the design of such a crop ideotype is the concept of a high Leaf Area Ratio of the stem, maximizing the leaf area per unit stem weight. This can be achieved principally through having small internodes, which subtend a proportionately large leaf. The simulation model developed predicts maximum root yields of approximately 30 t/ha/yr for plants with these characteristics (Cock *et al.*, 1979). El-Sharkawy (2003) indicated that observed productivity on a field scale confirms the validity of the simulated ideotype.

Later various other desirable features have been added and include: (1) higher photosynthetic rates; and (2) longer leaf life; (3) less sensitivity of stomata to VPD for conditions where soil water is not limiting; (4) enhancement of heliotropism to optimize light interception at low LAIs in the morning and afternoon and to reduce heat load on the leaves at midday. The effects of variation in leaf life and photosynthetic rate are shown schematically in **Figure 7**. Shorter plants with small internodes that enhance the stem LAR have also been suggested (El-Sharkawy and Cock, 1987b); however, this concept has certain dangers as very short plants may not compete well with weeds and may produce not enough high quality planting material.

General Principles of Optimizing Production Under Stress Conditions.

The cassava crop has evolved and has been selected to grow well under stress. It has long been cultivated in semi-arid areas with long periods of water stress; it is frequently grown in poor soils or as the last crop in rotation before returning to fallow, and with minimal use of chemical pesticides (Cock, 1985). The cassava plant has various mechanisms that allow it to maintain yield stability under stress conditions (Cock, 1987). In general, plants subjected to stress should be naturally more vigorous than those grown under non-stress conditions. As stress is imposed the top growth is normally reduced, and hence a plant type with a LAI suitable for near ideal conditions will be suboptimal under stress conditions (**Figure 8)**. The vigorous variety MMex 59 has a supra-optimal LAI under good conditions, which becomes optimal under stressed conditions, whilst MCol 22 performs excellently under good conditions but is not well adapted to, in this case, water stress (Connor *et al.,* 1981). This suggests that to obtain a more stable and reliable yield under variable stress conditions it may be advisable to err on the side of excessive vigor under optimal conditions, thus ensuring that in the case of stress a reasonable yield will be obtained (Cock, 1985).

A further attribute of cassava is the tendency when faced with stress to reduce growth and make the best use of the available resources. This is particularly true of water and nutrient stress. A direct result of this is that under stress conditions the proportion of biomass distributed to the roots increases. Consequently, it is not uncommon for stressed plants of vigorous varieties to produce more roots than unstressed plants ((**Figure 8**, see for example, Connor and Cock, 1981).

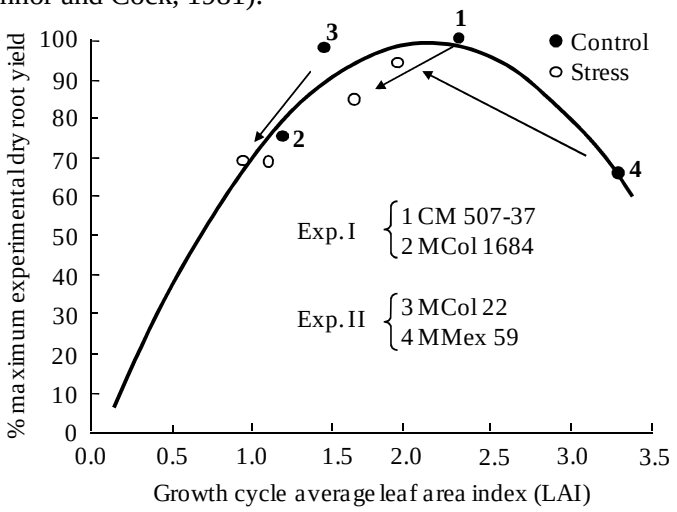

Figure 8. Root yield of four varieties presented as a percentage of trial maximum root yield as a function of LAI.
***Source**: Cock and El-Sharkawy, 1988a.*

Once established the cassava crop has no critical periods when stress can reduce yields to nothing. This is a common characteristic of crops that simultaneously produce leaves and stems as well as the economically useful part. Thus, for example, in a cereal crop a severe stress at anthesis may result in complete crop failure. This does not happen to cassava once it has been established as a crop. A frost or insect attack may completely defoliate the plant, or a prolonged drought may leave the crop totally without leaves. Nevertheless, when conditions improve the crop produces a flush of new leaves and plants continue to grow.

Diseases, pests and weeds

Weeds can be a major problem in a crop like cassava which has a slow initial development, and with optimal LAIs that may not be sufficiently high to shade out weed species. More vigorous clones may have lower yield potential than less vigorous types, but they tend to perform better when weed control is deficient (Cock, 1985).

A particularly difficult problem with weeds, and also with intercrops in certain circumstances, occurs with drought stress. As previously noted, cassava tends to conserve resources and use them efficiently when they are in scarce supply. Under drought conditions the presence of aggressive weeds or intercrops prevents the cassava crop from husbanding scarce resources. In the case of intercrops it would appear advisable to use short-cycle intercrops that can be harvested before drought stress occurs; or to use long-season crops with the same stomatal mechanisms as cassava to conserve water.

Diseases and pests may cause severe damage in cassava, but once again cassava has certain mechanisms which make it able to tolerate disease and pest attacks. With pests or diseases that attack the leaves or apices, more vigorous varieties are preferable. Removal of 50% of the leaves of a vigorous variety may actually increase the yield due to slower top growth, whereas similar defoliation of a less vigorous variety may reduce yields substantially (Cock, 1978). Similarly, removing a proportion of the apices to simulate pest damage increased the yield of a vigorous variety (Cock, 1978).

On the other hand, diseases or pests, such as for example spider mites, that reduce the photosynthetic rate of leaves over long periods are likely to cause severe damage. The solution to these types of pests is host plant resistance or other means of control. Cock (1978) concluded that cassava is relatively tolerant to disease and pest attacks due to the abundant opportunities to recover after damage. Relatively minor losses result from: (1) early death of some plants which is compensated by more vigorous growth of surrounding plants; (2) reduction in the number of active apices; (3) small decreases in thickened root numbers if they occur early in the growth cycle; and (4) small reductions in leaf size. On the other hand yields are markedly reduced when: (1) leaf life is reduced; (2) photosynthetic rate decreases substantially; (3) stems are severely damaged; and (4) there is a high percentage of early plant death or massive loss of active apices.

Drought stress

Cassava has many mechanisms that confer remarkable tolerance of drought. These mechanisms are so strong that, once established, it is extremely difficult to kill cassava plants through lack of water. Furthermore, not only will the plant survive but it will also produce relatively well under drought conditions. These mechanisms can broadly be divided into three main groups. Firstly, the crop reduces water use, thus conserving soil water; secondly, the limited amount of water consumed is used efficiently to produce biomass; and thirdly, the proportion of the biomass passing to the economically useful plant parts is increased.

The stomatal response to vapor pressure deficit (VPD) reduces the transpiration and photosynthesis of the crop when the VPD is large (**Figure 9**). This response is accentuated when there is a soil water deficit, but occurs even when plants are well watered. The photosynthetic water use efficiency (WUE) is greatest when the VPD is small. Cassava stomata, as in most crop plants, also close in the dark. Hence, the cassava crop, especially when there is a soil water deficit, tends to open its stomata and photosynthesize at those times when the VPD is small (early in the morning and to a lesser extent late in the afternoon) and the WUE is greatest. This mechanism enables the cassava crop to conserve soil water and only use it at those times when it can most efficiently convert solar energy, carbon dioxide and water into biomass. In contrast, crops such as maize and rice tend to maintain their stomata open and deplete the soil water rapidly when VPD is large until such a point comes when they wilt and even die due to the large negative water potential in their leaves. This stomatal response to VPD protects cassava from severe drought stress; unlike many other crops it is extremely difficult to kill cassava by subjecting it to drought conditions.

It is commonly observed that at the onset of the dry period cassava reduces its LAI and it is generally assumed that this decrease is due to more rapid leaf fall. In potted greenhouse plants leaf fall is indeed accelerated in stressed plants (Calatayud *et al.,* 2000a).

However, this does not appear to be the case in the field. The number of new nodal units produced and the size of their leaves is reduced under stress, but the longevity of those leaves still on the plant is little affected. In fact, it may even be increased (Connor and Cock, 1981). The reduction in the number of nodal units is principally due to reduced production of nodes per apex, but there is also a reduction in the forking and in the number of forks per branching point (Connor and Cock, 1981). The net effect of these processes is a reduction of the leaf surface from which transpiration can occur, and hence, reduced water consumption.

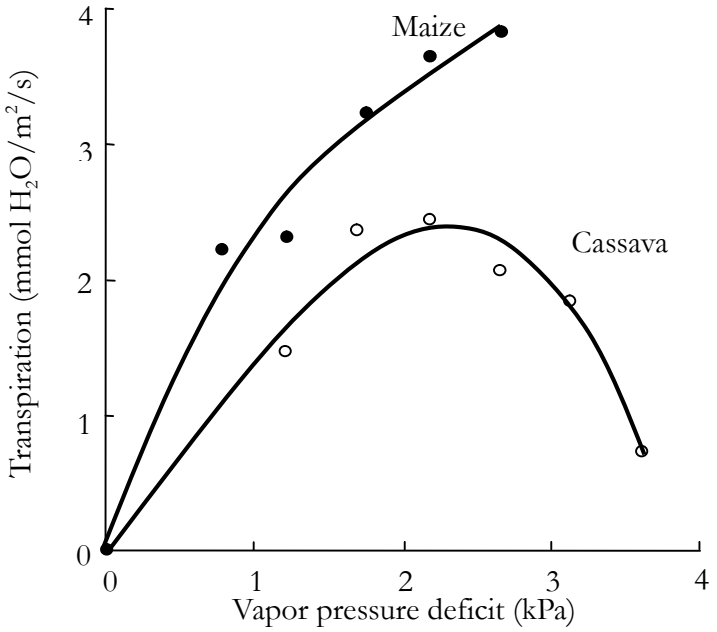

Figure 9. Transpiration as a function of vapor pressure deficit in cassava and maize.
***Source:** El Sharkawy et al., 1985.*

The cassava's heliotropic leaf movement is a mechanism for both protecting the leaves against excessive solar radiation by drooping of the younger leaves (Calatayud *et al.,* 2000), and also to maximize the interception of sunlight when VPD is low by tilting towards the light in the early morning and late afternoon (Berg *et al.,* 1986).

The smaller number of nodes produced under stress reduces the demand for substrate for the formation of stem tissue. The crop growth rate is also decreased under water stress due to both reduced photosynthesis when stomata close at large VPDs and also to the reduced leaf area, which reduces interception of solar radiation. The net effect of the reduced growth of stem tissue and reduced crop growth rate is an increase in the proportion of the biomass produced found in the roots. In vigorous varieties this effect can lead to stress plants yielding more than unstressed plants (Connor *et al.,* 1981; El-Sharkawy, 2006).

The root system of cassava is not particularly dense, but it is extensive. Cassava is capable of exploiting the available water to a depth of 2 m ore more (Connor *et al.,* 1981;

Keating *et al.*, 1982a). In a fairly typical soil the cassava plant can extract the equivalent of 160 mm of soil water during a drought period.

Table 2. Comparative water use efficiency of cassava, grain sorghum and field bean. Data in brackets is the harvest index measured as the percentage of dry economic yield over dry total biomass.

Species	Single leaf water use efficiency. (µmol CO_2/ mmol water)	Biomass of field-grown crops (g dry weight/kg water)	Economic yield (DM/kg water)
Cassava	5.3	2.9	1.7 (60%)
Sorghum	6.2	3.1	1.2 (40%)
Bean	3.5	1.7	0.7 (40%)
Cassava/sorghum (%)	85	94	140
Cassava/bean (%)	150	170	240

Source: *El-Sharkawy and Cock, 1986; El-Sharkawy, 2006.*

The combination of these mechanisms makes cassava an extremely water efficient crop, particular under conditions where evaporative demand is high. It is estimated that cassava uses about 270-300 kg water for each kg biomass produced, and 500 kg water per kg of economic yield which is comparable to such crops as sorghum which are renowned for their drought tolerance (**Table 2**) (El-Sharkawy, 2006).

The lack of a critical growth period when drought can cause complete crop failure is another important aspect of drought tolerance. After a prolonged dry period cassava rapidly recovers from drought. New nodal units are produced with leaves that may be even larger than those on unstressed plants. Photosynthetic rate of older leaves rapidly recovers and equals or exceeds the rate of plants that have not been stressed. The new flush of growth is supported by mobilization of root reserves to the leaves, the preferential sink, and root dry matter decreases in the short term (Lenis *et al.*, 2006). With the new flush of leaf growth the crop will continue to grow and produce at similar rates to plants that have not been subjected to stress. In summary, the drought tolerance of cassava is due to: (1) no critical period once the crop is established; (2) reduced leaf area formation with the onset of drought; (3) maintenance of leaves during the stress period; (4) reduced leaf area formation and increased distribution of biomass to roots; (5) stomatal closure when soil water is limited (stomata close before soil water is exhausted); (6) leaf movements and stomatal control to maximize photosynthesis when water use efficiency (WUE) is greatest; (7) leaf movements to reduce radiation load on leaves at midday; and (8) roots that slowly extend to absorb water down the soil profile. Furthermore, once the stress period ends and soil water is available the crop recovers rapidly by: (1) a new flush of leaves using root and stem reserves (starch content of roots decreases); and (2) renewed photosynthesis by the old leaves.

It is the combination of these remarkable mechanisms that has made cassava one of the best options to avoid famine and to provide a livelihood for those who live in areas of uncertain rainfall and drought.

Nutrient stress

Many crops show severe symptoms of major nutrient deficiencies. A rice or maize field with insufficient nitrogen can readily be distinguished by the yellowish tinge of the leaves. Similarly, phosphorous deficiency in many plants can be readily diagnosed from the red to purple coloring of the leaf borders. In greenhouses (Cruz *et al.,* 2003), and specially in controlled nutrient solution experiments, cassava plants can show major nutrient deficiency, but these are rarely observed in the field. Rather than growing continuously and ending up with low levels of nutrients in plant tissue, the cassava plant tends to reduce its growth according to the available nutrients. This is particularly true in the case of nitrogen (**Table 3,** Cock 1984) but appears to be less so in the case of phosphorous (Pellet *et al.,* 1993b) and potassium.

Table 3. Effect of high, medium, and low fertility levels on leaf area index (LAI) and nutrient concentration of leaves + petioles of MMex 59 six months after planting.

		Nutrient concentration (% of dry matter)			Nutrient concentration (mg/dm^2 leaf surface)		
Fertility level	LAI	N	P	K	N	P	K
High	5.29	3.69	0.25	2.00	18.9	1.28	10.3
Medium	3.54	3.68	0.19	1.40	20.2	1.04	7.7
Low	1.65	3.52	0.18	0.73	21.7	1.11	4.5

Source*: J.H. Cock and G. Parra (unpublished. data).*

Theoretically it is more efficient in terms of total biomass production to restrict leaf area and maintain a nutrient status of the leaves commensurate with a high photosynthetic rate, rather than distributing limited nutrients over a larger leaf area. Under nutrient stress cassava restricts the leaf area to maintain the nutrient concentration of the leaves (**Table 3).** The nutrient status of the leaves is sufficiently high to maintain the photosynthetic rate in the nutrient-stressed plants (De Tafur *et al.,* 1997) (**Table 4**), thus maximizing biomass production under limited nutrient supply.

The restriction of shoot growth due to nutrient stress changes the balance of biomass distribution in favor of the roots, increasing the harvest index of nutrient stressed plants. Hence, the reduction in root yield is less than that of biomass production, leading to a greater harvest index in stressed plants.

Table 4. Leaf photosynthetic rate (Pn) and LAI of fertilized and unfertilized cassava. The comparisons for statistical significance are between treatments for each variety.

Variety	Treatment	Pn (μmol/m^2/s)	LAI
MCol 1684	Fertilized	32.8 NS[1)]	1.76 a
	Not fertilized	30.7 NS	1.06 b
MCol 507-37	Fertilized	37.6 NS	1.36 a
	Not fertilized	35.3 NS	0.89 b

***Source**: De Tafur et al., 1997.*

Whilst cassava in general as a species produces relatively well when nutrients are limiting, there appear to be varietal differences in the ability to tolerate stress. At the CIAT Quilichao station, with very low levels of available soil P, more than 1600 germplasm accessions were screened for yield at low and moderate soil P levels. Several accessions were found to produce high yields at both the low and the moderate P level, suggesting that it should be possible to breed varieties that tolerate low soil P levels (El Sharkawy, 2003). Nevertheless, on extremely low phosphorous soils, well managed to maintain effective strains of mycorrhiza, cassava performs very well (Howeler *et al.*, 1982; Howeler and Sieverding, 1983; Howeler pers. comm.) suggesting that there may be no need to breed for low P tolerant varieties; it may simply be easier to manage the crop and the mycorrhiza.

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CHAPTER 4

BASIC CONCEPTS OF QUANTITATIVE GENETICS

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1. INTRODUCTION

Understanding the inheritance of agronomically relevant traits in cassava is fundamental for an efficient genetic improvement of the crop. Basic principles of qualitative genetics were established with the pioneering work of Mendel more than a hundred years ago. Later many scientists contributed to the elaboration of the principles of quantitative genetics which basically split it into three major components: additive, dominant and epistatic effects (or variances). This chapter provides an initial introduction to the concepts of quantitative genetics and its connection to crop breeding strategies. This chapter will be followed by a second chapter where experimental results are provided. In the analysis of genetic variation the pioneering research by Mendel focused on traits that segregated in contrasting classes (i.e. tall versus dwarf, purple versus white flowers, etc.). In fact, it was the sharp and distinctive phenotypic classes observed in these traits that helped Mendel to reach his breakthrough discoveries. The inheritance of these traits, identified as *qualitative*, is easy to study and predict because of the large effect of different alleles on the phenotype, which results in distinctive phenotypic classes, and because of the negligible effect of the environment in their expression.

There is, however, additional variation that was not originally addressed by Mendel, which is certainly less obvious and refers, for example, to the differences in plant height within the tall plants, or within the dwarf ones. This kind of variation does not result in clearly distinguishable classes but in a continuous variation between the extreme phenotypes and is, therefore, called *quantitative*. Quantitative traits are controlled by several genes (in this context "several" may mean as few as five genes, but generally refers to many more). The effect on the phenotype of the information contained at each locus is relatively small and, therefore, it is difficult to track them in segregating progenies. In addition, the environment frequently affects the expression of quantitative traits. It is important to emphasize that quantitative trait alleles are inherited and segregate according to Mendel's laws. The difference is that their individual segregation cannot be tracked based on the phenotypes.

The analysis of the inheritance of qualitative traits is relatively simple with obvious, clearly distinguishable contrasting phenotypes and negligible interaction with the environment. These traits are typically analyzed by determining the segregation ratios of the two or three classes that, for example, a single gene inheritance typically determines. On the other hand, understanding the mechanisms behind quantitative inheritance is much more complex because the segregation of individual alleles cannot be properly tracked, there are a large number of genes involved, there are interactions within and between loci and the environment confounds the expression of the trait under study.

G.V. Yule (1906), E.M. East (1908) and G.H. Shull (1909) first developed the principles of quantitative genetics in the early 1900s, at the dawn of the age of modern plant breeding. R.A. Fisher (1918) and S. Wright (1921) were key scientists to incorporate

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some of that new information on gene behavior early in the 20th century. In the ensuing years many scientists added to our understanding of quantitative genetics: Comstock, 1952; Comstock and Robinson, 1948; Falconer; 1981; Hallauer and Miranda, 1988; Hayman and Mather, 1955; Lynch and Walsh, 1998; and Mather and Jinks, 1977; and Vencovsky and Barriga, 1992. According to Lynch and Walsh (1998), the impact of early quantitative genetics theory influenced profoundly the evolution of modern theoretical and applied statistics, facilitating the developments of the theory behind regression and correlation analyses and the principles upon which the analysis of variance is based. A brief description follows of the most important concepts of quantitative genetics in relation to plant breeding.

2. ADDITIVE, DOMINANCE AND OVERDOMINANCE EFFECTS IN SINGLE-GENE INHERITANCE

A hypothetical model illustrates the different types of gene actions that will be used. **Figure 1** shows the three possible genotypes at a given locus and their respective phenotypes. The homozygous genotypes are identified as **aa** and **AA,** and the heterozygote as **Aa**. The phenotype of the heterozygous genotype (**Aa** = 18) is exactly halfway between the two values defined by the homozygotes. The mode of inheritance depicted in **Figure 1** is called *additive*. In our hypothetical situation, each dose of an "**A**" allele will add six units to the phenotypic expression of the trait. Hence the shift from genotype **aa** to **Aa** resulted in their respective phenotypes increasing from 12 to 18, and shifting from genotype **Aa** to **AA** also resulted in a phenotypic increase of six units.

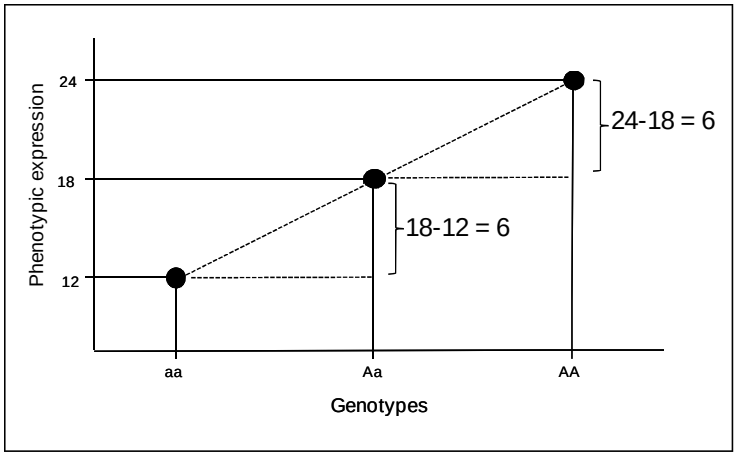

Figure 1. A hypothetical case of the relationship between genotypes and phenotypes for a trait whose inheritance is fully due to additive effects.

The situation illustrated in **Figure 2** is similar to that shown in **Figure 1** for the two homozygotes. The phenotypic value for **aa** is 12 and that of **AA** is 24. The phenotypic expression of the heterozygote (**Aa**), however, is identical to that of the homozygote **AA**. In the heterozygote, the allele **A** exerts a complete dominance over allele **a**, and therefore, genotypes **Aa** and **AA** express the same phenotypes. This is the typical situation analyzed by Mendel in his pioneering work and is known as **complete dominance**. The dominance

can be exerted either by the allele that increases the expression of the character or by the one that reduces it. The hypothetical model in **Figure 2** showed **A** dominating over **a**, but the opposite situation could have been chosen without affecting the conclusions.

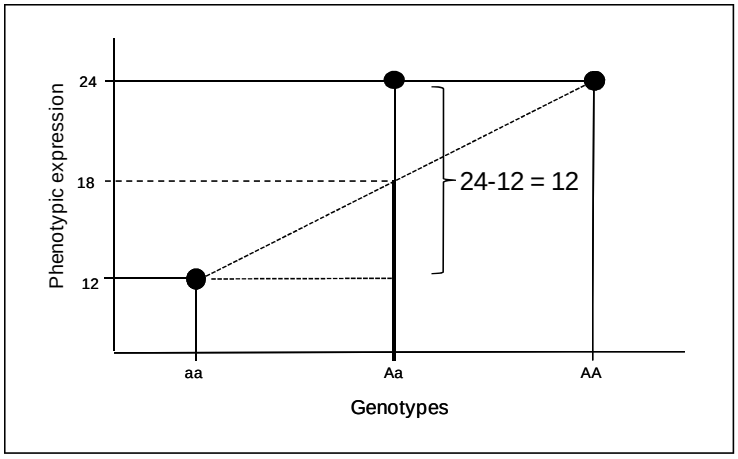

*Figure 2. A hypothetical case of the relationship between genotypes and phenotypes for a trait whose inheritance shows complete dominance of the allele (**A**) that increases the expression of the trait.*

The difference between actual and expressed value of the heterozygote, and the expected value in the additive model, is called the dominance deviation (**Figure 2**).

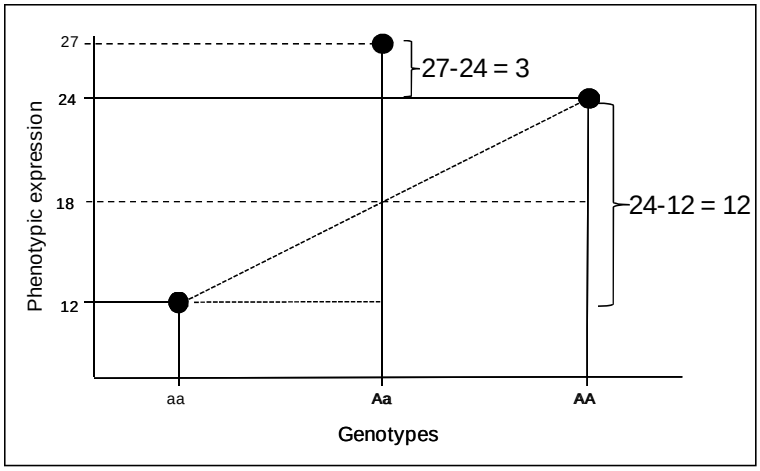

*Figure 3. A hypothetical case of the relationship between genotypes and phenotypes for a trait whose inheritance shows overdominance of the allele (**A**) that increases the expression of the trait.*

Finally, **Figure 3** illustrates another situation frequently observed in nature. In this case, the trait shows *overdominance*. The overdominance (or *transgresive*) inheritance is characterized by a heterozygote with a phenotype outside the range of variation defined by the two homozygotes. In our example, the range of variation defined by the two homozygotes was between 12 and 24), and the phenotype of the heterozygote was 27. Overdominance plays an important role in the heterosis or hybrid vigor shown by many crops, including cassava.

3. ADDITIVE, DOMINANCE AND OVERDOMINANCE EFFECTS IN A TRAIT CONTROLLED BY SEVERAL GENES

In the phenotypic expression of quantitative traits several genes segregate simultaneously and each of them may show any of the gene actions illustrated in **Figures 1** to **3**. In addition, a confounding effect can occur when the allele that reduces trait expression is dominant, while in other loci the opposite is true. Quantitative genetic analysis evolved with the purpose of explaining this type of situation, where:

- Several to many genes are involved.
- There are no clearly distinguishable phenotypic classes.
- There is strong genotype-by-environment interaction.

It soon becomes evident that the involvement of more than one gene in the expression of a given trait greatly complicates the analysis. Allard (1960) provided the example presented in **Table 1.** In the first model presented (additive) the phenotypic expression is defined by the number of capital letters present in the genotype (capital letters representing the allele that *increases* the phenotypic expression of the character). The contribution of **A** is slightly higher than that of **B**. It should be apparent that this model is very simple and, to a large extent, predictable. A key feature of the additive model presented in **Table 1** is that the substitution of one allele by another results in predictable increases or decreases in the phenotype and this is true regardless of the other genes present. In every case, when one allele **A** replaces another allele **a,** the phenotype increases by two units. Similarly, when one allele **B** replaces **b**, the phenotype increases one unit, regardless of the status in the locus **A/a**. Two important properties of this model are:

- The effect of replacing **a** by **A** (or **b** by **B)** is the same regardless if what happens in the homozygote or in the heterozygote. Dominance effects, therefore, are absent.
- The effect of replacing **a** by **A** (or **b** by **B**) is the same regardless of the status at the other loci. There is no interaction among loci, i.e. epistatic effects are absent.

Model II from **Table 1** illustrates the typical case of two dominant genes. Genotypes **AA** and **Aa** have the same phenotype, in contrast with that of **aa**. The same can be seen with genotypes **BB** and **Bb**, whose phenotypes are identical but differ from that of **bb.** Although the model introduces some changes in relation to the simple additive model, the relationship between genotype and phenotype is still relatively simple and predictable:

- The effect of replacing **a** by **A** (or **b** by **B)** is different depending on the circumstances. If the replacement occurs from **aa** to **Aa** (or from **bb** to **Bb)** there is a drastic effect on the phenotype. If the replacement occurs from **Aa** to **AA** (or from **Bb** to **BB)**, on the other hand, there is no effect.
- The effect of replacing alleles in loci **A/a** or in loci **B/b** is the same regardless of the status at the other loci. Epistatic effects, therefore, are still absent.

Model III in **Table 1** introduces an additional complexity. There are only two phenotypes possible: those that have at least one capital letter allele at each of the two loci and those that have capital letter alleles at one or no locus. In this model the individual effect of alleles present in locus **A/a** cannot be determined unless there is information about the status of locus **B/b**. This is the typical case of *complementary gene action*, which is one of the simplest epistatic effects observed in nature. In spite of the dependency of the genotype at one locus on other loci, the relationship between phenotype and genotype is still relatively simple and predictable.

Table 1. Alternative hypothetical models for the segregation at two loci [1)].

I. Additive model				**II. Dominance model**			
AABB	AABb	AAbb	**AA--**	AABB	AABb	AAbb	**AA--**
7	6	5	**6**	4	4	2	**3½**
AaBB	AaBb	Aabb	**Aa--**	AaBB	AaBb	Aabb	**Aa--**
5	4	3	**4**	4	4	2	**3½**
AaBB	aaBb	aabb	**aa--**	aaBB	aaBb	aabb	**aa--**
3	2	1	**2**	3	3	1	**2½**
--BB	--Bb	--bb		--BB	--Bb	--bb	
5	4	3		3¾	3½	1¾	
III. Complementary epistasis				**IV. Complex epistatis**			
AABB	AABb	AAbb	**AA--**	AABB	AABb	AAbb	**AA--**
3	3	1	**2½**	4	2	3	**2¾**
AaBB	AaBb	Aabb	**Aa--**	AaBB	AaBb	Aabb	**Aa--**
3	3	1	**2½**	4	3	1	**2¾**
AaBB	aaBb	aabb	**aa--**	aaBB	aaBb	aabb	**aa--**
1	1	1	**1**	3	2	1	**2**
--BB	--Bb	--bb		--BB	--Bb	--bb	
2½	2½	1		3¾	3½	1½	

[1)] Numbers indicate the genotypic value for each genotype. The border rows and columns represent the mean genotypic values for the three conditions possible at each locus (assuming a gene frequency of ½ at each locus).
***Source**: adapted from Allard, 1960.*

The complications derived from epistatic effects are more clearly illustrated in Model IV from **Table 1**. The first column for Model IV (**BB** in every case) illustrates full dominance of **A** for the different allelic combinations for locus **A/a**. The second, column (**Bb** in every case), however, shows over-dominance with the heterozygote **Aa** having a higher phenotypic expression than both homozygotes. Finally, the third column (**bb** is common in the three genotypes) illustrates full dominance of **a,** for the different allelic combinations for locus **A/a**.

Segregation at locus **B/b** (when state of locus **A/a** is constant**)** results in a different set of reactions. The first row always has genotypes **AA--**, and in this case segregation at locus **B/b** shows *under-dominance*. In the second row (all genotypes **Aa--**), segregation at the **B/b** locus reveals *partial dominance* and in the third row (all genotypes **aa--**) a completely additive gene action. Many examples of epistatic relationships between genes in different loci have been reported. An interesting review, which is relevant to cassava, illustrates the complexities derived from the interaction between different genes involved in the synthesis of the two polymers present in starch: amylose and amylopectin (Jobling, 2004).

The situations illustrated above led Fisher (1918) to propose the three main gene action effects that are the subject of quantitative genetics studies:

- *Additive variance or effects* were initially defined as the differences between the homozygotes, but in genetic designs are generally related to the breeding value of an individual, which is described below.

- *Dominance variance or effects* are basically derived by the interactions among alleles in the same locus (intra-allelic interaction).

- *Epistasis variance or effects* are associated with interactions among alleles at different loci (inter-allelic interaction).

These gene actions can then be summarized in a classical formula for partitioning genetic variance in its components as follows:

$$\boldsymbol{\sigma^2_G = \sigma^2_A + \sigma^2_D + \sigma^2_{AA} + \sigma^2_{AD} + \sigma^2_{DD} + \sigma^2_{AAA} + \sigma^2_{AAD} + \sigma^2_{ADD} + \sigma^2_{DDD} + \ldots etc.}$$

where:

σ^2_G = Total genetic variance
σ^2_A = Additive genetic variance (associated with breeding value)
σ^2_D = Dominance genetic variance
σ^2_{AA} = Digenic epistatic variance between additive effects
σ^2_{AD} = Digenic epistatic variance between additive and dominance effects
σ^2_{DD} = Digenic epistatic variance between dominance effects
σ^2_{AAA}, σ^2_{AAD}, σ^2_{ADD} and σ^2_{DDD} = Trigenic epistatic variances among different effects.

4. ADDITIVE EFFECTS, GENERAL COMBINING ABILITY AND BREEDING VALUE

A major constraint in quantitative genetics is the impossibility for tracking individual alleles. As complete as the model developed by Fisher was, it remained a theoretical development with little practical relevance until the concepts of *breeding value* and *average effect of gene substitution*, were defined (Falconer, 1981).

The average effect of gene substitution is closely associated with the additive model. In the first example of **Table 1** (additive model) the average effect for the gene substitution of **a** by **A** is two units. In effect, replacing one **a** allele by the **A** allele in the homozygote **aa--**, results in the heterozygote whose average phenotypic value has shifted from 2 to 4 (see right column for that model). Similarly, replacing the **a** allele in the

heterozygote **Aa--** by an **A** allele, results in the **AA--** homozygote and a phenotypic increase, again, of two units (from 4 to 6). For locus B/b the same trend can be observed, with the only difference that allele substitutions results in smaller phenotypic changes (1 unit for each allelic substitution).

If all the genes affecting a quantitative trait were considered, the average effects of all the alleles present in a given progenitor would determine the mean genotypic value of its progeny (Falconer, 1981), which is directly related to the concept of *breeding value*. The breeding value of a given progenitor is defined by the average effects of the alleles it possesses. The relevance of these concepts will now be explained. Is should be clear that the average effects of the many alleles involved in the inheritance of a given trait cannot be measured. On the other hand, the breeding value of that progenitor can in fact be measured through the mean performance of the progeny it produces. In practical terms the breeding value of an individual is related to a better-known parameter: general combining ability.

The breeding value of an individual relates to the relative performance of the progeny that it generates. When an individual is randomly crossed with a large number of mates from the same reference population, the breeding value of that individual will be twice the average deviation of its progeny from the population mean. The variation in breeding values has been associated with the additive effects of genes, as they were described above, although strictly speaking they are not the same. A major advantage of the breeding value is that it can actually be measured.

By the middle of the 20^{th} century most of the principles of quantitative genetics had been laid. Many different articles lead to the demonstration that, if certain conditions were met, the genetic variation in a given population could be partitioned into its additive and dominance components using different family structures. One major limitation in these studies is the frequent assumption that epistasis (between different loci) is negligible.

Several genetic designs have been developed to measure the relative importance of additivity, dominance and epistasis in the expression of different traits, in view of the information provided in **Table 2.** Generation mean analysis (Mather and Jinks, 1977) is a design favored by breeders and geneticists working with self-pollinated species. Diallel crosses and North Carolina Designs I and II are the most common approaches used in cross-pollinated crops (Hallauer and Miranda, 1988). Depending on whether genetic effects are considered *random* or *fixed*, the studies will focus on variances or effects. Regardless of the kind of study, a common assumption in most of these studies is the absence of epistasis in the expression of the traits analyzed.

Most of the designs listed above focus on the between-family variation. The within-family variation is seldom analyzed because it does not provide any relevant additional information. Cassava and other crops with vegetative propagation, however, have the advantage that individual genotypes can be cloned. By cloning, the within-family variation can be partitioned into its genetic and environmental components. Moreover, the interaction between genetic and environmental components of variation can also be measured. This is a decided advantage given the large proportion of the total genetic variance that generally remains in the within-family component (**Table 2**) and because in doing this the relative importance of epistasis can be measured indirectly (as will be shown later in this chapter).

Genetic studies analyzing the importance of epistatic effects are not very common, particularly in annual crops. Adequate measurement of epistasis for complex traits, such as yield, is difficult and expensive. Reports on the relevance of this kind of gene action are infrequent and have generally taken advantage of the vegetative multiplication that some species offer (Comstock *et al.*, 1958; Stonecypher and McCullough, 1986; Foster and

Shaw, 1988; Rönnberg-Wästljung *et al*., 1994; Rönnberg-Wästljung and Gullberg, 1999; Isik *et al*., 2003). Many of these reports are on forest trees. Because of the complexities of these analyses and the costs involved, the scarce reports in the literature on epistasis are frequently based on a limited sample of genotypes, which consequently may result in contradictory or unreliable results.

Table 2. Distribution of the genetic variation into its additive and dominance components in a population with different family structures.

	Between families		Within families	
Type of family	σ^2_A	σ^2_D	σ^2_A	σ^2_D
Half-sib families	1/4	0	¾	0
S_1 families from half-sibs	3/8	0	5/8	0
S_2 families from half-sibs	7/16	0	9/16	0
Full-sib families	1/2	1/4	1/2	3/4
S_1 / F_3	1	1/4	1/2	1/2
S_2 / F_4	3/2	3/16	1/4	1/4
S_3 / F_5	7/4	7/64	1/8	1/8
S_4 / F_6	15/8	15/256	1/16	1/16
S_5 / F_7	31/16	31/1024	1/32	1/32
S_6 / F_8	63/32	63/4096	1/64	1/64
…	…	…	…	…
S_∞ / F_∞	2	0	0	0

***Source**: Hallauer and Miranda, 1981; Venkovsky and Barriga, 1992.*

5. RELEVANCE OF THE COMPONENTS OF GENETIC VARIANCE TO CROP BREEDING

At the beginning of this chapter we mentioned that knowledge about the inheritance of traits is fundamental for efficient and effective genetic improvement of crops. After this description of the different components that make up the total genetic variance, we turn to a discussion of the implications that this information has for breeding in general and for cassava in particular.

A very important concept that needs to be developed now is the relationship between genetic effects and the way they are transmitted to the progenies. Additive gene effects can be transmitted to the progeny because they depend on the alleles that a given individual possess. However, non-additive genetic effect (dominance and epistasis) depend on specific allelic ***combinations*** (intra-locus in dominance and inter-loci in epistasis) and these allelic *combinations* can not be transmitted to the progeny. When there is sexual reproduction the progenitor produces gametes that are a recombination of the genetic make-up of the individuals involved and only one copy of each allele (in case of diploid inheritance) are transmitted to the progeny through each gamete. Taking the information presented in **Table 1**, a double heterozygote (AaBb) will generate nine different genotypes (AABB, AABb, AAbb, AaBB, AaBb, Aabb, aaBB, aaBb and aabb) and depending on the mode of gene action, these nine genotypes will generate from two to nine different phenotypes. The specific genetic conditions that lead to the occurrence of a particular phenotype that depends in dominance and epistatic effects (for example in loci A and B in the double heterozygote AaBb) **cannot** be transmitted to the progeny. This is true because

AaBb individuals will produce gametes that will be AB, Ab, aB or ab. It should be clear that the dominance effects cannot be transmitted to the progeny because only one allele per locus (of the two possible alleles used in this example) is present in the gamete (either A or a; and B or b). Epistatic effects cannot be transmitted to the progeny either. The independent assortment of alleles in meiosis will randomly allocate allele A with either B or b (in equal proportions). Similarly, only 50% of the gametes carrying allele B will also carry allele A. The other 50% of gametes with allele B will carry allele a. It is impossible, therefore, to guarantee that the double heterozygote (AaBb) will produce only gametes AB. The only situation where the A and B alleles would be transmitted together is when they are closely linked. This occurs when two loci are very closely together in the same chromosome and therefore, the chance of independent assortment is greatly reduced or null.

Now, the concepts presented above do not mean that the double heterozygote cannot be produced again. The self-pollination of the double heterozygote will randomly produce an average of 25% of the progeny with the AaBb genotype. In practice, the maize hybrid industry will develop inbred lines (for example AAbb and aaBB) that, when crossed, will generate 100% of AaBb progeny. But this approach requires a special breeding approach and the incorporation of inbreeding that will allow the exploitation of dominance and epistatic effects in a controlled fashion.

Additive genetic effects, by definition, are related to the breeding value of an individual when used as progenitor in a breeding nursery. Breeding value is closely related to the mean performance of a progeny of a given parent, compared with the overall average performance across the progenies of many progenitors. Additive effects are relatively simple to estimate and to improve, since most breeding schemes will properly exploit them. The main concern that a breeder should have is that enough additive variation is available for success. Additive effects (or variance) has been redefined so it can be measured by the most common quantitative genetics designs and it is directly associated with general combining ability (GCA) effects. While it may appear to be a contradiction, complete dominance gene action can strongly influence GCA effects. In this case, however, a single dominant gene does not produce a truly quantitative segregation.

The dominance effects associated with heterosis (or hybrid vigor) are more typically those in which many genes are involved in the control of the trait. Epistasis and dominance are frequently grouped together and renamed as the “non-additive” fraction of the genetic variance. As mentioned above, dominance and epistasis represent the within- and the between-loci interactions, respectively. The successful exploitation of these “non-additive” effects requires a special breeding scheme. Several such schemes, known as reciprocal recurrent selection methods, have been developed and successfully used in maize breeding (Hallauer and Miranda, 1988; Pandey and Gardner, 1992). A common feature of the different schemes is the presence of two (or more) heterotic or reciprocal populations. The goal of reciprocal recurrent selection is to increase the performance *per se* of the populations and, more importantly, of the crosses among them. In theory these schemes tend to improve the ‘*complementarity*’ of the two reciprocal populations, in such a way that when they are crossed the number of heterozygous loci is maximized. Heterozygosity, as explained above, is responsible for the heterosis or hybrid vigor observed in many plant species.

The breeding schemes used for improving additive and non-additive traits should be different. The breeding value (or general combining ability) of a given parent depends on the genes it contains (as well as the frequency of these genes in the reference population). Additive effects depend on “good genes” that can be properly identified and, more importantly, transmitted to the progeny. On the other hand, non-additive effects (or

variances) depend on specific gene combinations, which cannot be transmitted, as such, to the progeny when sexual reproduction is involved. The gametes (pollen and ovules) can transmit genes but not gene combinations. Therefore, good gene combinations need to be reconstituted every time there is sexual reproduction. Reciprocal recurrent selection can exploit genetic effects that depend on gene combinations (dominance effects *within* the loci and epistasis effects *between* loci) because it facilitates the reconstitution of desirable gene combinations after sexual recombination has taken place.

Furthermore, given the length of each recurrent selection cycle in cassava, and the total number of years involved, it is also convenient to introduce inbreeding in the process. Current breeding systems rely on the crosses among predominantly heterozygous parents. Use of inbred parents would result in the gradual fixation (cycle after cycle of selection) of the appropriate genes in the complementing inbred materials in such a way that the gradual and consistent improvement of gene combinations becomes feasible. In other words, the respective inbred lines can be further improved (as is the case in the industry of hybrid maize) by crosses with related lines that generate restricted genetic variation in the resulting progenies.

The reduced variation allows for the improvement of the parental lines as progenitors (further enhancing their '*complementarity*' in the crosses they generate). Because parents are inbred and they are only crossed with related lines, some loci will already be at the homozygous status in both lines and the system allows this to continue this way while generating limited genetic variation. This is important because it allows the gradual improvement of the cross between two progenitors so, cycle after cycle, they can produce better gene combinations. It is important that the inbred parents are crossed with related lines to maintain many loci at the homozygous stage and generate variation in just a few loci. Otherwise the desirable fixed gene effects would be quickly lost. The entire process described above is to improve the parental lines so, when they are crossed with lines from the reciprocal population they produce an outstanding hybrid. This is the hybrid that the farmer will plant and multiply vegetatively as it is ordinarily done in cassava.

Figure 4 illustrates the advantages of reciprocal recurrent selection, particularly when inbred lines are involved. The process may start with the formation of two *heterotic populations* (A and B) that complement each other well. If no such a case is found, the populations can be defined based on other criteria. One approach could be to use genetic distances determined by molecular markers. Inbred lines are derived from each population. In the process, selection for good agronomic performance, for example, plant type and resistance to pests and diseases may be exerted. The segregation may also allow for the identification of useful recessive traits, particularly for starch properties (e.g. waxy starch), nutritional characteristics, modified plant-type or disease/insect resistance.

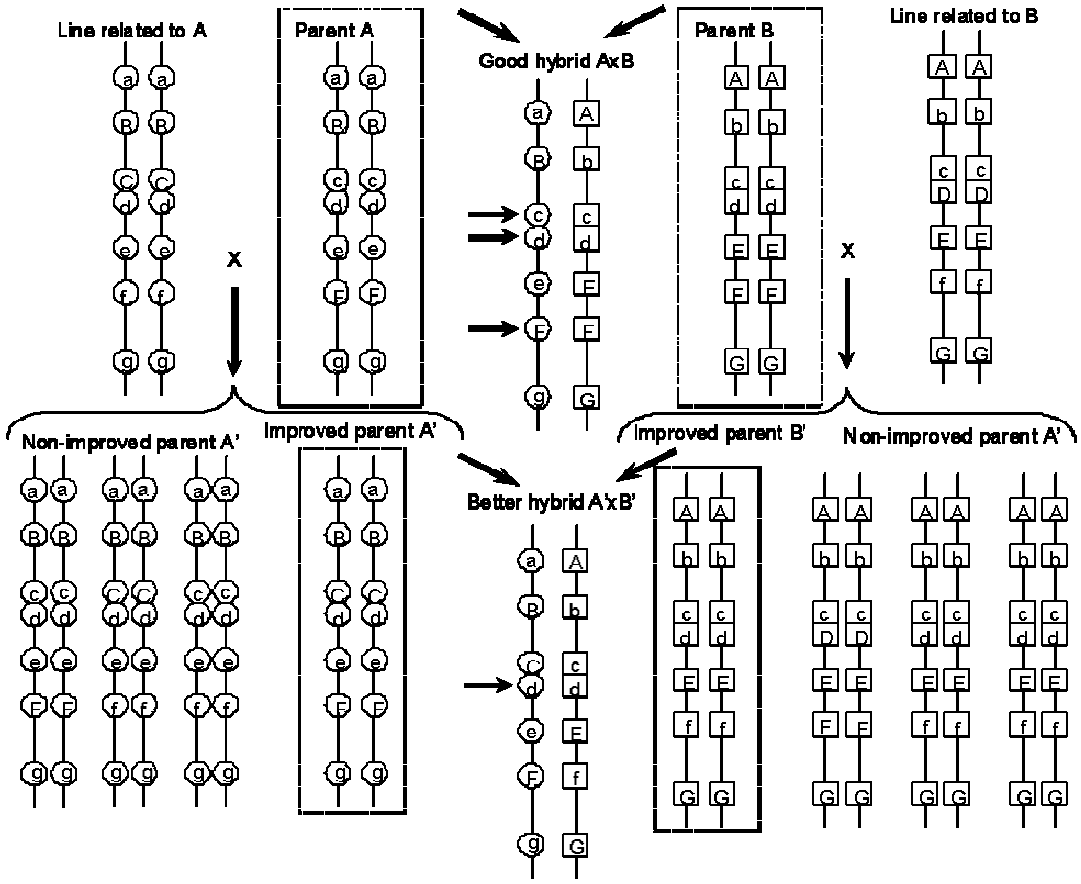

Figure 4. Reciprocal recurrent selection based on the development of inbred parental lines. Parents A and B belong to different reciprocal populations. The hybrid they produce is outstanding. Parent A is crossed with a related line to produce progenies that segregate only for a restricted number of loci. Parent B is also crossed with a related line. As a result of the hybrid produced by the improved versions of parents A and B shows better performance than the original hybrid. Genetic progress is more directed, consistent and predictable.

The inbred lines from population A are crossed with inbred lines from population B. Eventually a pair of lines will be identified because of the outstanding hybrid they produce (AxB). The hybrid may be released to farmers who will multiply it taking advantage of the vegetative multiplication in cassava. The fact that this hybrid is the result of the cross between two inbred lines offers additional advantages. The inbred lines can be stored and/or shipped to other cassava-breeding projects as botanical seed. The hybrid can be reconstructed each time the same inbred parents are crossed. Therefore, tissue culture approaches to clean the planting materials after several cycles of vegetative reproduction, can be overcome by this simple procedure.

Moreover, improved gene combinations can be obtained to produce a better hybrid than the original AxB cross. Inbred line A is crossed with a related line from the same population and, in the process, a segregation restricted to a limited number of loci will occur. The same is done with inbred line B. It is important that a limited number of loci segregate because the original AxB hybrid is already an excellent cultivar and it is desirable not to loose the good gene combinations that it possesses. In this particular case (which is greatly simplified), there are three loci in the AxB hybrid that are not in the heterozygote conditions (loci **cc**, **dd** and **FF**). It has been demonstrated that hybrid vigor depends largely on a large number of loci at heterozygous state (Crow, 1999); therefore additional breeding of the inbred lines should focus (in our example) on establishing distinct states for these three loci in the two parent lines, such that the F_1 is as nearly completely heterozygous as possible.

Several inbred lines are obtained from each population hoping to produce genotypes that will be better parents than the two lines originally used to produce the hybrid AxB. In the lower half of **Figure 4** the segregation of inbred lines from populations A and B is depicted. Among these lines, two showed a better performance, when crossed, compared with the original hybrid. As a result only locus **dd** remains in a non-heterozygous condition: a subject of interest for a new cycle of selection that could eventually solve this remaining undesirable situation. It is obvious that this scheme is ideal for gradually and consistently fixing desirable allelic combinations. In the process the individual alleles cannot be tracked and the whole process is done "blindly" through phenotypic evaluations of the resulting hybrids. This scheme has been used successfully by different hybrid maize companies and has resulted in constant genetic gains in maize genetic productivity during the last 70 years (Duvick, 1984).

It must be emphasized that the scheme described above cannot be implemented in cassava today, because the production of inbred progenitors is cumbersome and difficult from the logistic point of view. However, CIAT has been working for several years (in collaboration with colleagues from a few National Agriculture Research Institutions and Universities) to develop protocols for the production of doubled-haploids, which by definition are fully homozygous. Doubled-haploids can be generated through a diversity of tissue culture techniques (anther, microspore and/or ovule culture) or doing wide crosses using distant relatives or unrelated plant species (e.g. *Ricinus communis*).

6. HERITABILITY

In the previous section the impact of the environment in the expression of a given trait or characteristic was not considered. Environment and genotype-by-environment interaction, however, play an important role in the expression of many traits with agronomic relevance. The recognition that not all the variation that can be observed (*phenotypic* variation) is due to genetic effect leads to the distinction between *phenotype* and *genotype*. Phenotype is the observable manifestation of a specific genotype grown

under specific conditions. Genotype is the consolidation of all the genes that characterize an individual. A genotype is the genetic constitution of an organism as opposed to its physical appearance or phenotype. The relationship between phenotype and genotype leads to the concept of heritability, which is another important parameter in quantitative genetics and plant breeding. Broadly speaking heritability can be defined as the proportion of the phenotypic variance that can be explained by genetic effects:

$h^2 = \boldsymbol{\sigma^2_G / \sigma^2_P}$
where:

σ^2_G = Total genetic variance
σ^2_P = Phenotypic variance

The phenotypic variance can be partitioned into genetic variance (σ^2_G), environmental variance (σ^2_E), the interaction between these two sources of variation (σ^2_{G*E}) and the experimental error (σ^2_{Ex}). Therefore another way to describe "broad sense" heritability is:

$$h^2 = \boldsymbol{\sigma^2_G / [\sigma^2_G + \sigma^2_{En} + \sigma^2_{G*E} + \sigma^2_{Ex}]}$$

The most important use of the concept of heritability is for understanding the best breeding approaches. Genetic gains are directly related to heritability. The higher the heritability, the higher (or faster) the genetic gains will be. For example, an area of research where breeders will have a large opportunity to increase heritability is reducing the environmental (σ^2_E) and genotype-by-environment (σ^2_{G*E}) variances. A simple way to reduce the environmental variance is selecting a uniform plot to conduct the evaluation and adequate evaluation locations. The experimental error is a consolidation of many different uncontrolled sources of variation, such as inaccuracies in a scale, within plot variation, biases arising from sampling procedures, mistakes in data recording or logging, etc. A good breeder will make sure that the σ^2_{Ex} is minimized. The formula provided above is known as 'broad sense' heritability and tends to overestimate genetic gains because not all the genetic effects can be transmitted to the progeny (as explained above at the beginning of Section 5). Only additive effects can be transmitted to the progeny by a given individual. Therefore, breeders and geneticists find the 'narrow sense' heritability more useful:

$$h^2 = \boldsymbol{\sigma^2_A / [\sigma^2_G + \sigma^2_E + \sigma^2_{G*E} + \sigma^2_{Ex}]}$$

The main difference between broad and narrow sense heritability estimates is the numerator of the formula. In narrow sense heritability the additive genetic variance (σ^2_A) is used so it provides information regarding the degree to which a phenotype can be modified by selection and breeding.

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CHAPTER 5

CASSAVA GENETIC IMPROVEMENT

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1. REPRODUCTION IN CASSAVA.

Cassava can be propagated either by stem cuttings or by sexual seed. However, the former is the most common practice used by farmers for multiplication and planting purposes. Propagation from true seed occurs occasionally in farmers' fields and, as such, is a starting point for the generation of useful genetic diversity (Alves, 2002). Most breeding programs generate seed through crossing, as a mean of creating new genetic variation. Occasionally botanical seed has also been used in commercial propagation schemes (Iglesias *et al.*, 1994; Rajendran *et al.*, 2000).

Cassava is monoecious, with female flowers opening 10-14 days before the male ones on the same branch. Self-pollination can occur because male and female flowers on different branches or on different plants of the same genotypes can open simultaneously (Jennings and Iglesias, 2002). Flowering depends on the genotype and the environmental conditions. Branching occurs when an inflorescence is formed. Because erect, non-branching types, are frequently preferred by farmers, the crossing of elite clones in certain regions may become more difficult because of the scarcity of their flowers. Synchronization of flowering remains a difficult issue in cassava breeding. Some clones flower relatively early at 4 or 5 months after planting, whereas others flower only at 8 to 10 months after planting. Because of this, and the time required for the seed to mature, it takes generally no less than a year to obtain seeds of a planned cross. On average, between one and two seeds (out of the three possible formed in the trilocular fruit) per pollination are obtained. Several publications illustrate the procedures for controlled pollinations in cassava (Jennings and Iglesias, 2002; Kawano, 1980). Seeds often have a dormancy period for a few months after maturity, and they require relatively high temperatures (30-35ºC) for optimum germination (Ellis *et al.*, 1982).

2. BREEDING OBJECTIVES

As is the case for many other crops a key objective in most cassava-breeding projects is high and stable production of fresh roots. The reliability and resilience of the crop is one of the characteristics of cassava most valued by farmers. However, breeding objectives will depend heavily on the ultimate use of the crop. Productivity plays a major role in industrial uses of cassava (i.e. starch production and dried roots for animal feed), whereas stability of production will be fundamental in the many regions where cassava is the main subsistence crop. Industrial uses of cassava require not only high productivity of fresh roots, but a minimum level of dry matter content in these roots. This additional requirement arises from the fact that the starch industry would produce larger amount of effluent liquids if dry matter content is low in the roots. Similarly, drying yards would require an additional day or two to complete the drying process. In areas where cassava is important for human consumption, cooking quality or starch characteristics may be more

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important than productivity. Consumers frequently associate good cooking quality with other morphological traits such as the color of the peel of the roots as 'markers'. Farmers frequently reject any change in such morphological traits, although they may have little or no correlation with actual cooking quality. Because of those types of farmers and consumer preferences, participatory research and breeding approaches had to be developed for cassava breeding (DeVries and Toenniessen, 2001; Gonçalvez Fukuda *et al.*, 2000; Gonçalvez Fukuda and Saad, 2001). Other root quality traits relevant to different cassava breeding programs of the world are the cyanogenic potential in the roots (Dixon *et al.*, 1994), early bulking capacity, higher protein content in the roots and reduced post-harvest physiological deterioration. Unfortunately, the genetic variability for the latter two traits is limited in *M. esculenta* and, therefore, inter-specific crosses with other *Manihot* species to introgress useful alleles have been attempted (Ceballos *et al.*, 2006a; 2007a). The more recent market for the production of bio-ethanol from cassava roots has opened new requirements that may be linked to the costs of transforming the fresh or dry roots into ethanol (Reddy *et al.*, 2008).

2.1 Tolerance or resistance to pests and diseases

High and stable productivity relies heavily on adaptation of different crops to biotic and abiotic stresses. In Asia, cassava has developed considerably because of the relatively 'healthy' environment. Few diseases have been reported to cause serious economic damage in Asia, with the exception of the cassava mosaic disease present in India and Sri Lanka. In other continents, however, the situation is different. In Africa Cassava Mosaic Disease (CMD) and Cassava Brown Streak Disease (CBSD) are important constraints (Calvert and Thresh, 2002). A disease similar to CMD is also present in southern India. In certain regions of Latin America and the Caribbean (LAC), Frogskin Disease causes roots to become "corky" and commercially unusable. The causal agent has not yet been identified, although it has been suspected for many years that it may be a virus. Bacterial blight, induced by *Xanthomonas axonopodis* pv. *manihotis* (also known as *X. campestris* pv. *manihotis*), is found in Asia, Africa and LAC, and can have devastating effects on yield and the availability of planting material, particularly in Africa and LAC (Hillocks and Wydra, 2002). Several fungal diseases also may affect cassava productivity. Super-elongation disease, induced by *Sphaceloma manihoticola* (Teleomorph: *Elsinoe brasiliensis*) is widespread in the Americas, from Mexico to Southern Brazil. *Phoma* species cause leaf and stem lesions in the tropical highlands. Several species of *Phytophthora* induce root rot, but also different species of the genera *Sclerotium, Armillaea* and *Fusarium*. There are sources of genetic resistance to most of these diseases (CIAT, 2001; Hillocks and Wydra, 2002).

In the case of Asia, pests may be more damaging to cassava than diseases. Several arthropod pests feed on cassava and can reduce productivity. *Tetranychus* spp and other red mite species (from genera Eutetranychus and Oligonychus) are the most conspicuous problem in Asia, whereas in other regions of the world it is the green mite (*Mononychellus tanajoa*) that can devastate cassava fields (Bellotti *et al.*, 2002; Nyiira, 1975). The mealybugs *Phenacoccus manihotis* and *P. herreri* feed on cassava fields of Africa and LAC, respectively, but the presence of one of these two species is becoming common in cassava fields of Thailand. Thrips (particularly *Frankliniella williamsi* and *Scyrtotrips manihoti*) considerably reduce yields of susceptible genotypes. Clones with pubescent

leaves in their early stages of development offer excellent levels of resistance to these insects (Belloti, 2002), and this trait has been broadly incorporated into improved varieties.

Whiteflies are among the most widespread pests in cassava. *Aleurotrachelus socialis* is the predominant species in northern South America, where it causes considerable crop damage through direct feeding. *Bemisia tabaci* is widely distributed in tropical Africa and several Asian countries. The major effect of *B. tabaci* is as a vector of the devastating CMD disease in Africa. Several other species of whiteflies affect cassava in different regions, but *Aleurodicus dispersus* is probably the most common in Asia. Genetic resistance to whiteflies in cassava has been found particularly for *A. socialis* in several germplasm accessions from the CIAT collection (Bellotti, 2002). Based on breeding work at CIAT, Colombia released the first whitefly-resistant variety of any crop.

There are several other arthropod pests affecting cassava roots, foliage and/or stems, particularly Lepidoptera, Diptera and Hemiptera. There is little or no genetic resistance to those pests and their management is commonly achieved through biological control measures. Attempts to produce transgenic cassava have succeeded with the introduction of *cry* genes encoding insect-specific endotoxins (Bt toxins) from *Bacillus thuringiensis* (Fregene and Puonti-Kaerlas, 2002; Taylor *et al.,* 2004). The recent establishment of SIBS-ETH Shanghai Center for Cassava Biotechnology at the Institute of Plant Physiology and Ecology of the Chinese Academy of Sciences has brought new capacities in the area of genetic transformation. Several attempts are currently underway to produce transgenic cassava with different traits, such as insect resistance or leaf retention for increased tolerance to drought (Zhang Peng *et al.,* 2008).

2.2 Tolerance to abiotic stresses

There are a variety of abiotic factors limiting cassava productivity. The crop is frequently grown in drought-prone regions and/or on low fertility soils. It can also be found in alkaline or acidic soils, most frequently the latter. Some traits associated with adaptation to these conditions have been suggested (Jennings and Iglesias, 2002), such as: leaf longevity (Lenis *et al.,* 2006), optimum Leaf Area Index, and ideal plant architecture (Hanh *et al.,* 1979; Kawano *et al.,* 1998; Kawano 2003). The capacity of the stems to withstand long storage periods (sometimes up to two months) from harvest to planting affects final density of established plants and is an important trait for areas with relatively long dry spells or erratic rainfall, because the storage period may extend to the point it compromises their viability. While there is known genetic variation for stem storability, it has not been a major breeding objective of any program so far.

A serious constraint to cassava production is the short shelf life of its roots due to post-harvest physiological deterioration (PPD). PPD begins within 24 hours (Beeching *et al.,* 1998; Rickard, 1985) and rapidly renders the roots unpalatable and unmarketable. Consequently, cassava roots need to be consumed soon after harvesting (Van Oirschot *et al.,* 2000). The short shelf-life severely limits the marketing options because it increases the likelihood of losses, marketing costs, and access to urban markets is limited to those close to the production sites.

PPD begins with vascular streaking, which is a blue-black discoloration of the xylem parenchyma, followed by general discoloration of the storage parenchyma. Five to seven days later microbial activity causes further deterioration. Additionally, respiration is induced (Hirose *et al.,* 1984) resulting in starch hydrolysis (Uritani *et al.,* 1984). The

processes involved in PPD resemble typical changes associated with the plant's response to wounding that triggers a cascade of biochemical reactions, which are frequently oxidative in nature (Beeching *et al.,* 1998; Hirose *et al.,* 1984; Uritani *et al.,* 1984). Specific genes involved in PPD have been identified and characterized, and their expressions evaluated (Reilly *et al.,* 2001). Handling and storage conditions of the roots affect the speed and magnitude of PPD. Keeping roots at 10°C and 80% air relative humidity delays the onset of PPD by two weeks. Maintaining roots in controlled atmosphere conditions also delayed the onset of PPD (Zapata, 2001). The anti-oxidant properties of carotenoids pigments have also been found to delay the onset of PPD (Sánchez *et al.,* 2005).

2.3 Addressing the needs of different industries

During the 1990s there was a drastic change in the economies of tropical and subtropical countries where cassava is grown. As a result of the globalization of the economies, it became obvious that tropical production of maize was not competitive compared with that from temperate regions. Several factors explain this situation (Pandey and Gardner, 1992); there has been an increased volume of temperate maize imported by tropical countries. This, in turn, has opened an opportunity that was never available to cassava before because both governments and private sectors now realize that the crop is a key but underutilized commodity (Ceballos *et al.,* 2004). These changes made clear that, in addition to high and stable productivity, the cassava-breeding project had the opportunity of expanding and exploiting genetic variability that would generate clones with increased value for the different industrial processes where cassava can be a strategic raw material. Examples of key traits for the different industries are mentioned below:

Animal Feed: Cassava is an important commodity as a source of energy in animal diets. However, it has low levels of protein and, therefore, its use imply the need to modify the composition of the diet with an additional source of protein (typically soybean derivatives). For this reason, the rule of thumb says that the price of cassava cannot be more than 70% of the price of maize (Tewe, 2004). Key qualitative traits for this industry would be finding cassava clones with higher levels of proteins in the roots (Ceballos *et al.,* 2006b). In addition, the possibilities of other nutritional traits such as pro-vitamin A carotenoids would be beneficial (Chavez *et al.,* 2005).

Starch Industry: Cassava starch has properties of its own which make it particularly adapted (or not adapted) to certain uses. This sector has always requested novel cassava starch types to diversify its uses.

Ethanol and bioplastics: This is a relatively new demand for cassava products, which was accentuated with the recent increases in the price of oil. A "*sugary*" cassava, such as one recently reported (Carvalho *et al.,* 2004), would make the process of fermentation to produce ethanol or lactic acid (an alternative product in the pathway for the production of bioplastics) economically and environmentally less expensive.

Processed food*:* Acyanogenesis (roots without even traces of cyanogenic glucosides) has been a trait requested by this sector.

3. PRE-BREEDING IN CASSAVA.

As in most crop breeding activities, cassava genetic improvement starts with the assembly and evaluation of a broad germplasm base, followed by production of new recombinant genotypes derived from selected elite clones. Scientific cassava breeding began only a few decades ago and, therefore, the divergence between landraces and improved germplasm is not as wide as in crops with a more extensive breeding history. As a result, landrace accessions play a more relevant role in cassava than in other crops. For example, Nanzhi 199, a very popular variety grown in the Guangxi province of China, is actually a landrace (MPan 19) from the germplasm collection at CIAT. Parental lines are selected based mainly on their *per se* performance and little progress has been made to use general combining ability or breeding value (Hallauer and Miranda, 1988) as a criteria of parental selection. Crossing can be by controlled pollinations, done manually, to produce full sib families or else in polycross nurseries where open pollination results in half-sib families.

Genetic variability available within *Manihot* has not been fully explored and screened. This genetic wealth has not been fully exploited and, therefore, should offer interesting possibilities for the future. In part, the limited evaluation of cassava genetic variability is because the collection and maintenance of cassava germplasm is difficult, cumbersome and expensive. Furthermore, detection of some of the economically important traits in the roots is more difficult. For instance, the many different starch mutants in maize (popcorn, sweet, floury, waxy corn, etc.) are easily recognizable. No equivalent mutant had been reported for cassava until recently.

Nutritional quality factors studied to date also show relatively low genetic variation, with the exception of the high carotene levels found in yellow cassava roots (Iglesias *et al.*, 1997). However, as a result of new initiatives, an aggressive screening of cassava germplasm allowed Chávez *et al.,* to report in 2005 not only interesting variation in carotenoids, but also for crude protein content in the roots. Further analyses (Ceballos *et al.,* 2006b) have confirmed the occurrence of cassava clones with 2-3 times higher crude protein contents (≈6-8%) compared with the typical levels found in cassava roots (≈2%).

Another activity that is relevant to the proper screening of genetic variability is the introduction of inbreeding (see Chapter 6 on Heterosis and Inbreeding), which allows for the identification of useful recessive traits. CIAT started to systematically self-pollinate cassava germplasm (elite improved clones and materials from the germplasm collection) in 2004. As a result, in early 2006 two interesting mutations were found (Ceballos *et al.*, 2007b; 2008) in a self-pollinated plant that possesses a waxy starch (reduced proportion of amylose) in its roots. This discovery is important not only because of the economic value of such a trait, but also because it proves the usefulness of introducing inbreeding in cassava genetic improvement.

Pre-breeding activities also include wide crosses with wild relatives of cassava (Blair *et al.*, 2007). Several traits of commercial relevance have been found in these wild relatives and introgressed into the cassava gene pool. Among the most relevant ones are the tolerance to PPD in *M. walkerae;* increased protein content in *M. tristis* and *M. peruviana;* resistance to the cassava green mite in *M. esculenta* sub spp. *flavellifolia* and amylose-free starch in *M. crassisepala* and *M. chlorosticta*. *M. glaziovii* is suspected to be the origin of resistance to cassava mosaic disease and to the hornworm in segregating progenies from crosses which involved this species as one of the progenitors (Blair *et al.,* 2007). Improved

nutritional quality has also been reported in wild relatives of cassava by Nassar and Ortiz (2008).

4. BREEDING SCHEME

For open pollinations a field planting design developed by Wright (1965) is followed to maximize the frequency of crosses of all the parental lines incorporated in the nursery. Knowledge about flowering capacity is important in order to select a group of materials with synchronized flowering. When there are considerable differences in flowering habit a delayed planting and/or pruning of the earliest flowering genotypes may be required. At harvest, the seeds harvested from each clone are bulked to form a half-sib family. Seeds from full-sib families can be obtained in isolated open pollination plots were two clones are planted together and one of them, chosen to act as female progenitor is emasculated. Alternatively, several male-sterile clones have been identified, which can act as female parents.

Different crossing schemes are used to produce botanical seed in cassava (Kawano, 1980). At CIAT, seeds are germinated under greenhouse conditions and the resulting seedlings transplanted to the field when they are about 20-25 cm tall. Root systems in plants derived from botanical seed or vegetative cuttings may differ considerably. The taproots from seedlings tend to store fewer starches than roots from cuttings (Rajendran *et al.,* 2000). Because of this, it is difficult, if not impossible, to correlate the root yield of clones at later stages in the evaluation/selection process with early results from the plants obtained from botanical seeds (Morante *et al.,* 2005). However, when seeds are germinated in containers and later transplanted, the taproot often does not develop, and the seedling-derived plant may be more similar to subsequent stake-derived plants in terms of starchy root conformation.

The multiplication rate in cassava (based on vegetative cuttings) is low. Under good environmental conditions a cassava plant from a modern clone can easily yield up to 20 cuttings. However, when thousands of clones are handled under non-optimal conditions, which are the typical target environments for most cassava-breeding projects, a realistic multiplication rate will range only from 5 to 10. This imposes a critical limitation because it takes several years until enough planting material is available for the multi-location trials. One further complication in a cassava program is the number of factors that can affect quality of planting material. For example, the original positioning of the vegetative cutting along the stem affects considerably the performance of the plant it originates. Cuttings from the mid-section of the stems usually produce better performing plants than those at the top or the bottom. This variation in the performance of the plant depending on the physiological status of the vegetative cutting, results in larger experimental errors and undesirable variation in the evaluation process.

Table 1 illustrates the old and new selection scheme used by the cassava-breeding project at CIAT. It begins with the crossing of elite clones and ends when a few clones surviving the selection process reach the stage of regional trials across several locations. There is some variation among different cassava-breeding programs, regarding the numbers of genotypes and plants representing them through the different stages (Ceballos *et al.,* 2007a); however, the numbers presented in **Table 1** are fairly common and illustrate the different stages required to complete a selection cycle and the kind of selection pressures generally applied (Ceballos *et al.,* 2004; 2007a).

The first selection is conducted the second year on the nurseries with plants derived from botanical seed (F1 in **Table 1**). Because of the low correlations between the performance at this early stage of selection and when the genotypes reach replicated trials, the early selections are based on high-heritability traits such as plant type, branching habits and, particularly, reaction to diseases (Hahn *et al.*, 1980a, 1980b; Hershey, 1984; Iglesias and Hershey, 1994; Morante *et al.*, 2005). The second stage of selection is called Clonal Evaluation Trial (CET). The few surviving genotypes from the single-plant selection conducted during the F_1 stage produce the 6-10 vegetative cuttings required for this second step. The capacity to produce this number of cuttings is in fact another selection criteria utilized at the F_1 stage. CETs usually range from 2000 to 3000 clones. Within a given trial, however, the same number of plants is used to avoid the confounding effects between number of plants and genotypic differences. Because the competition between neighboring genotypes in the CET may favor more vigorous plant architectures, selection at this stage relies heavily on high heritability traits such as harvest index (Kawano *et al.*, 1998; Kawano, 2003; Morante *et al.*, 2005). Plant type is an important selection criterion at early stages of selection: plants whose main stem does not branch until it reaches about 1 m are preferred (Kawano *et al*., 1978; Hahn *et al.*, 1979). Other selection criteria at this stage include high dry matter and low cyanogenic potential (Iglesias and Hershey, 1994). Between 100 and 300 clones survive the CET. A common feature in the first two stages of selection for most programs is that selection is frequently visual with no data recording, in order to manage a larger number of materials at lower costs.

Table 1. Description of the previous and current evaluation and selection stages utilized in cassava for a given target environment.

Time [1)]	Previous evaluation scheme [2)]	Time [1)]	Current evaluation scheme [2)]
0-6	**F1**: 4000 recombinant seeds germinated. Two stakes taken.	0-12	**F1**: 4000 recombinant seeds germinated. Eight stakes taken.
7-18	**F1-C1**: 4000 genotypes: 1 plant in target environment, 1 in Palmira	13-24	**CET**: 2000 clones, 8-plant plots, 1 replication, 1 location.
19-30	**CET**: 1500 clones, 6-plant plots, 1replication, 1 location.	25-36	**PYT**: 200 clones, 10-plant plots, 3 replications, 1 location.
31-42	**PYT**: 120 clones, 20-plant plots, 1 replication, 1 location	37-48	**AYT**: 60 clones, 25-plant plots, 3 replications, 1-2 locations.
43-54	**AYT**: 40 clones, 25-plant plots, 3 replications, 1-2 locations	49-60	**AYT1**: 60 clones, 25-plant plots, 3 replications, 2-4 locations
55-66	**AYT1**: 30 clones, 25-plant plots, 3 replications, 1-4 locations	61-72	**RT**: 15 clones, 25-plant plots, 3 replications, 6 locations.
67-78	**RT**: 15 clones, 25-plant plots, 3 replications, 6 locations.		

[1)] Time in months after germination of the recombinant botanical seed.
[2)] CET (clonal evaluation trial); PYT (preliminary yield trial); AYT (advanced yield trial), RT (regional trial).

One important trait that makes the harvest of large trials such as the CET expensive and time demanding is the measurement of dry matter content (DMC) in the roots. The productivity of cassava depends ultimately on the amount of fresh roots produced and the DMC of those roots. It is feasible to have excellent dry matter yields based on high production of fresh roots, even if they have below-average DMC. This situation is generally not acceptable because the transport and processing costs are too high. A sample of about 5 kg of roots is weighed in a hanging scale and then, the same sample of roots, is weighed with the roots completely immersed in water. The relationship between the two weights provides an accurate estimate of DMC. A simple modification of the system provided large benefits reducing the time required to quantify DMC. A few years ago weighing of roots in the water was made with a three-beam scale that required about one minute per sample. In 2000 the use of an electronic scale that requires only a few seconds to stabilize was introduced. Furthermore, since the electronic scale does not require any intervention, two scales can be set up, so while one is stabilizing the operator records the reading from the other scale (Ceballos *et al.*, 2007a). This has improved considerably the number of clones that can be evaluated and reduced the costs of harvest and selection of CETs.

The following stage of selection, the preliminary yield trials (PYT), at CIAT are currently based on the evaluation of 10 plants in three replications. The ten plants in each replication are planted in two 5-plant rows. Rows are spaced at only 0.8 m instead of the standard 1.0 m, and one empty row is left between plots to increase within clone competition and reduce between clone competition. Large genetic variability occurs among clones, even from the same family. Although poor performing clones are mostly eliminated at the CET stage, there is still a considerable variation in the PYT trials. This highlights the need for a gradual process of selection and the need to avoid strong selection pressures.

With the initiation of replicated trials the emphasis of selection shifts from high-heritability traits to those of low heritability such as yield (Morante *et al.*, 2005). Starting with PYT and increasingly during the Advanced Yield Trials (AYT) and the Regional Trials (RT) there will be a greater weight on yield and its stability across locations. Cooking quality, “poundability” (IITA), and “farinha” quality (Brazil) trials will also begin at these stages, when the number of genotypes evaluated has been reduced to a manageable size. AYT are typically grown in 1-2 locations for two consecutive years. They have three replications per location and plots are four rows with five plants per row. Yield data is taken from the six central plants of the plot and the remaining 14 plants are used as source of planting material for the next season. RTs are conducted for at least 2 years in 4-10 locations each year. Plots have five rows with five plants per row. Yield data is taken from the nine central plants.

The clones that show outstanding performance in the RTs are released as new varieties and, often, incorporated as parents in the crossing nurseries. This completes a selection cycle and a new one begins. It should be pointed out that the selection scheme described above has the following characteristics:

- The process is indeed a mass phenotypic recurrent selection, because no familydata are involved in the selection process.

- Few data are taken in early stages of selection, especially on genotypes that can be readily discarded by visual evaluation. Therefore, no data regarding

general combining ability effects (≈ breeding value) are available for a better selection of parental materials.

- There is no proper separation between general (GCA ≈ additive) and specific (SCA ≈ heterotic) combining ability effects. The outstanding performance of selected materials is likely to depend substantially on positive heterotic effects, which cannot be transferred to the progenies sexually derived from them.

- Inbreeding has been intentionally omitted in the breeding scheme. Therefore large genetic loads are likely to remain hidden in cassava populations, and useful recessive traits are difficult to detect.

- Two or more stages of selection may be based on non-replicated trials. A large proportion of genotypes are eliminated without the proper evaluation set up.

Because of the foregoing reasons there are some clear opportunities to further improve the efficiency and effectiveness of cassava breeding. Kawano *et al.* (1998) mention that during a 14-year period, about 372,000 genotypes, derived from 4,130 crosses, were evaluated at CIAT-Rayong Field Crops Research Center in Thailand. Only three genotypes emerged from the selection process to be released as official varieties. Nonetheless, it should be mentioned that these varieties have achieved remarkable success in Asia, with more that one million hectares planted. Similar experiences have been observed at IITA, CIAT-Colombia and Brazil. The resulting increases in productivity account for a higher income (about one billion US $ annually) to the poor farmers who grow the improved germplasm (Kawano, 2003).

4. APPROACHES TO IMPROVE THE EFFICIENCY OF CASSAVA BREEDING

4.1 Stratification of selection in the large Clonal Evaluation Trials

A major problem with the CET is its large size (easily 2 ha in size) and the unavoidable environmental effect in the selection. This problem is particularly relevant in the case of cassava, because the target environments for cassava are typically in “marginal” agriculture conditions and prone to large variation. Since CETs are frequently the first stage of evaluation, only a few stakes (< 10) are available for trials. So the introduction of replications that could help to overcome this problem is not practical.

The same simple principles suggested by Gardner in 1961 were recently introduced for the evaluations of CETs. The plot where the CET is going to be planted is divided in three “blocks” of about equal size trying to maximize differences among blocks and minimize variation within each block. The replication of each clone is difficult to implement because of the lack of enough planting material available for CETs. On the other hand, clones are grouped in either full- or half-sib families. Since many clones are generally available from each family they are randomly allocated in one of these three “blocks”. In other words instead of planting all the clones from a given family together, one after the other, they are split into three groups; these are planted in the three blocks into which the entire evaluation has been divided (**Figure 1**). This approach allows for two interesting advantages:

There is a replication effect for the families because all the clones from a given family are scattered in three "repetitions" in the field. The averages from all these clones are less affected by the environmental variation in such a large experiment.

Selection is made within each block. This is similar to the stratified mass selection suggested by Gardner. This approach effectively overcomes the environmental variation that can be measured by comparing the means of each block.

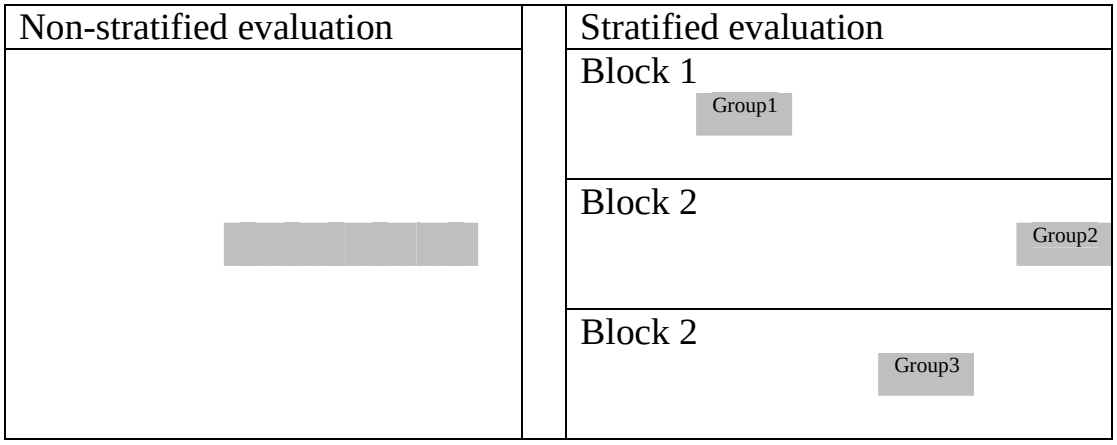

Figure 1. Illustration of a clonal evaluation trial (CET) where all the members of a given family were planted together in a non-stratified field layout (left). On the right there is a scheme of the way the CET could be stratified. All the families in the CET are equally divided in groups of approximately the same size (in the example there are three groups). The field where the CET is going to be planted is divided in as many blocks as groups each family was divided into. Each group is randomly allocated in these blocks and planted. If selection of the best performing clones is conducted ***within*** *each block, the environmental difference between the three blocks does not affect the selection, thus improving the efficiency.*

Because all the clones from the CET are divided, the average performance of each family can be estimated more precisely, because each family is scattered in three different parts of the field, whereas before it was concentrated in just one sector (**Figure 1**). As a consequence, the estimates of GCA for each family (described in detail below) is much more precise.

A summary of the results from the CET harvested in 2003 for the three main target environments (Sub-Humid, Acid Soils and Mid-Altitude Valleys) is presented in **Table 2**. The benefit of the introduction of stratified selection is directly proportional to the differences between the mean performances in each of the strata. In general, variations in the order of 10-20% have been observed among the average performances of the three blocks. This is, in other words, the gain in the precision attained by introducing the stratification of the CETs. Currently the possibility of increasing the number of blocks to four or five is under consideration.

4.2 Estimation of breeding values from Clonal Evaluation Trial data

One of the major decisions taken by any breeder is the selection of parents used to produce a new generation of segregating progenies. In cassava, this decision has been mainly based on the *per se* performance of each clone. Nonetheless, some empirical knowledge about the quality of progenies produced by different parents could be produced. This lack of organized information on the breeding values of parental lines used in the breeding projects was partially due to the fact that no data was taken and recorded during the first stages of selection (CET and PYT in **Table 1**) or else, the data was incomplete. Therefore, it was not possible to generate a balanced set of data that would allow the breeder to have an idea of the relative performance of the progeny of each elite parental line. In other words, no formal process to assess the breeding values of the progenitors used in the cassava-breeding projects was available.

Table 2. Results of the *Clonal Evaluation Trials* for the three main target environments harvested in May 2003. Data present the variation between the three blocks into which each CET was divided.

Block	Yield (t/ha)		Harvest Index (0 to 1)[1)]	Plant type (1 to 5)[2)]	Dry matter content (%)	Selection Index[3)]
	Fresh roots	Dry matter				
Averages of the 412, 412 and 411 clones in Blocks 1, 2 and 3, respectively, from the CET targeting the acid-soil savannas						
Block 1	20.88	6.66	0.50	3.33	31.59	0.00
Block 2	21.73	6.88	0.49	3.35	31.24	0.00
Block 3	22.30	7.28	0.50	3.48	32.44	0.00
Averages of the 749, 746 and 705 clones in Blocks 1, 2 and 3, respectively, from the CET targeting the sub-humid conditions						
Block 1	14.19	3.70	0.50	2.87	26.09	0.00
Block 2	14.37	3.91	0.46	2.88	27.21	0.00
Block 3	12.89	3.38	0.44	2.87	26.26	0.00
Averages of the 605, 588 and 568 clones in Blocks 1, 2 and 3, respectively, from the CET targeting the mid-altitude valleys						
Block 1	24.05	8.86	0.63	2.68	36.61	0.00
Block 2	28.08	10.21	0.57	2.63	36.02	0.00
Block 3	27.51	9.76	0.54	2.97	35.09	0.00

1) The harvest index is obtained by dividing the production of commercial roots by total biomass (roots + aerial parts). Preferred harvest indexes are > 0.5.

2) Plant type integrates under one value, plant architecture, leaves health, and capacity to produce stakes on a scale, where 1 = excellent and 5 = very poor, is used.

3) Average selection index within blocks must be zero, because it is based on a combination of standardized variables.

To overcome this problem the decision was taken to record data and to introduce the use of selection indexes. Selection is made within each stratum as explained in the previous section. Data from each family is then pooled across the three blocks in which it was planted. The stratification means that, in a way, there is a replication effect at the family level. Since a given progenitor may be used more than once, data from all the families in which each progenitor participated are pooled together to obtain an idea of the general performance of all the progenies from a given parental clone.

Results from the CET harvested in 2003 for the sub-humid environment have been chosen as an example of the kind of information that the current evaluation system allows. These results are summarized in **Table 3**. A total of 39 parents participated in generating all the progenies evaluated in that CET. Some parents were used considerably more than others, to a large extent because of their flowering habit in Palmira where the crosses are made. MNGA 19 and SM 1433-4 were used as parent in 215 and 213 clones, respectively. On the other hand, SM 1657-14 and SM 1754-21 were the parents of only 21 and 28 clones, respectively.

Table 3. Number of progenies evaluated and selected from each progenitor. Data from the *Clonal Evaluation Trial* for the sub-humid environment (Santo Tomás, Atlántico Department, Colombia) harvested in 2003.

	Progenitor	Family size	Progenies selected (number)	Progenies selected (%)		Progenitor	Family size	Progenies selected (number)	Progenies selected (%)
1	R 90	73	45	61.6	21	CM 4365-3	41	4	9.8
2	KU 50	64	30	46.9	22	SM 1657-14	21	2	9.5
3	MTAI 8	73	34	46.2	23	SM 1210-10	83	7	8.4
4	R 5	32	13	40.6	24	SM 1201-5	37	3	8.1
5	SM 1068-10	68	20	29.4	25	SM 1422-4	51	4	7.8
6	SM 2192-6	50	12	24.0	26	CM 7389-9	103	8	7.8
7	SM 1411-5	97	23	23.7	27	SM 1521-10	42	3	7.1
8	CM 7514-8	118	24	20.3	28	SM 1754-21	28	2	7.1
9	SM 1657-12	52	10	19.2	29	SM 1210-10	101	7	6.9
10	SM 643-17	32	6	18.8	30	SM 1619-3	29	2	6.9
11	MVEN 25	53	9	17.0	31	CM 8027-3	46	3	6.5
12	SM 1665-2	57	9	15.8	32	MNGA 19	215	12	5.6
13	CG 1141-1	33	5	15.2	33	CM 2772-3	28	1	3.6
14	SM 1511-6	87	13	14.9	34	SM 1600-4	61	2	3.3
15	SM 890-9	69	10	14.5	35	CM 7395-5	42	1	2.4
16	SM 1433-4	213	26	12.2	36	SM 805-15	73	1	1.4
17	SM 1565-17	108	13	12.0	37	CM 6438-14	53	0	0.0
18	CM 3372-4	52	6	11.5	38	CM 7514-7	56	0	0.0
19	CM 6754-8	49	5	10.2	39	SM 1431-2	33	0	0.0
20	SM 1438-2	109	11	10.1	**Average**				**13.6**

The interesting information from **Table 3** comes from the proportion of clones selected from each half-sib family. For instance the four best parents, regarding the proportion of their progenies being selected, were Rayong 90, KU50 (Kasetsart University 50), Rayong 60 (or MTAI 8) and Rayong 5. All of these clones were developed in Thailand and show excellent adaptation to the sub-humid environment of Colombia. More than 40% of the progenies from each of these parents were selected (**Table 3**). On the other hand, none of the progenies from CM 6438-14, CM 7514-7 and SM 1431-2 were selected although they were not particularly small families (53, 56, and 33 clones, respectively).

This system allows not only to know the proportion of clones derived from a given parent that has been selected. Since there is recorded phenotypic data for each genotype in the CET, an average across all the progenies from a given progenitor for all the variables is

available. It is possible, therefore, to find out which progeny tends to have above average fresh root yield or to conclude that the progeny from CM 6438-14 had an unacceptably low DMC (21.9%). This information is very valuable for defining which parents should stay in the crossing blocks, which should be removed, and also to suggest crosses that may result in better progenies because the breeder can complement better their advantages and defects of the various progenitors

4.3 Use of molecular markers

A molecular genetic map has been developed for cassava (Fregene *et al.,* 1997). Cassava genetic improvement can be made more efficient through the use of easily assayable molecular genetic or DNA markers (MAS) that enable the precise identification of genotype without the confounding effect of the environment; in other words, increasing heritability. MAS can also contribute to the efficient reduction of large breeding populations at the seedling stage based upon a 'minimum selection criteria'. This is particularly important given the length of the growing cycle of cassava and the expenses involved in the evaluation process. Therefore, a pre-selection at the F_1 phase could greatly enhance the efficiency of the CET trials. The selection of progenies based on genetic values derived from molecular marker data substantially increases the rate of genetic gain, especially if the number of cycles of evaluation or generations can be reduced (Meuwissen *et al.,* 2001).

Another application of MAS in cassava breeding is in reducing the length of time required for the introgression of traits from wild relatives. Wild relatives are an important source of genes for pest and disease resistance in cassava (Chavarriaga *et al.,* 2004; Blair *et al.,* 2007), but the need to reduce or eliminate undesirable donor genome content, linkage drag, can lengthen the process, making it unrealistic for most breeders. Simulations by Stam and Zeven (1981) indicate that markers could reduce linkage drag and would reduce the number of generations required in the backcross scheme. Hospital *et al.* (1992) corroborated this in achieving a reduction of two backcross generations with the use of molecular marker selection. Frisch *et al.* (1999), through a simulation study, found that the use of molecular markers for the introgression of a single target allele saved two to four backcross generations. They inferred that MAS had the potential to reach the same level of recurrent parent genome in generation BC_3 as reached in BC_7 without the use of molecular markers. Below a few examples of the use of molecular markers implemented for cassava genetic improvement are mentioned.

Selection for Casssava Mosaic Disease: An ideal target for MAS is breeding for disease resistance in the absence of the pathogen. This is the case of the cassava mosaic disease (CMD) in the Americas, where the disease does not occur. Molecular markers that allow selection of segregating progenies that carry the resistance of CMD have been successfully utilized (Blair *et al.,* 2007).

Introgression of useful traits from wild relatives: Wild *Manihot* germplasm offer a wealth of useful genes for the cultivated *M. esculenta* species, but their use in regular breeding programs is restricted due to linkage drag and the long reproductive breeding cycle. However, the use of molecular markers can facilitate the introgression of a single target region (Frisch *et al.,* 1999). It has been shown in several crops that the "tremendous genetic potential" locked up in wild relatives can be released more efficiently through the

aid of new tools of molecular genetic maps and the advanced back cross QTL mapping scheme (ABC-QTL). CIAT is currently implementing a modification of ABC-QTL to introgress genes for high protein content, waxy starch, delayed PPD, and resistance to whiteflies and the hornworm.

Mutation Breeding and DNA TILLING: The Targeted Induced Local Lesions in Natural Genomes (TILLING) is a technique based on single nucleotide polymorphism (SNP) detection by hetero-duplex analysis using the nuclease *Cel I* (McCallum *et al.*, 2000). The National University of Colombia and CIAT have initiated in 2004 a collaborative project on mutation breeding and DNA TILLING to identify novel starches, including waxy starch. More than 2000 sexual seeds were irradiated using gamma rays (a Cobalt-60 source) and fast neutrons at the International Agency of Atomic Energy (IAEA), Vienna, Austria, and shipped back to CIAT. The seeds were germinated, transferred to the field and over 1000 genotypes were self-pollinated (to overcome the problem of chimeras present in the M_1 generation) and produced more than 3000 S_1 plants. Work will focus on starch biosynthetic genes: the granule bound starch synthase (GBSSI) for the production of amylose, and the soluble starch synthase genes (SSSI, SSSII, SSSIII), for the production of amylopectin. DNA TILLING will be applied to these plants to detect sense point mutations and deletions in these genes.

Estimation of average heterozygosity during inbreeding of cassava: Cassava genotypes are heterozygous and very little inbreeding has been practiced until now in cassava breeding. But inbred lines are better as parents because they do not have the confounding effect of dominance and carry lower levels of genetic load (undesirable alleles). Essential aspects of inbreeding heterozygous crops is the starting point, represented by the average heterozygosity of the original parental lines, the homozygosity level of the selected genotypes at the end of the self-pollinating phase, and the speed of the self-pollination process (Scotti *et al.*, 2000). It is expected that the first few cycles of self-pollinations will result in a marked reduction of vigor (inbreeding depression associated with the genetic load of the parental lines). Therefore, selection for tolerance to inbreeding depression must be exerted. However, this selection is biased by the differences in homozygosity levels of segregating partially inbred genotypes. This highlights the need for a method to measure the heterozygosity level in these partially inbred individuals to be used in a co-variance correction in the selection of phenotypically vigorous genotypes. Eventually, molecular markers can also be used for determining regions in the genome that are particularly related to the expression of heterosis and for measuring genetic distances among inbred lines to direct crosses with higher probabilities of high heterosis.

Other Potential MAS targets: There are several additional activities based on molecular markers for the genetic improvement of cassava. The most important of these activities relates to the delineation of heterotic groups, and β-carotene content, cyanogenic potential, and dry matter content in the roots.

4.4 Genetic transformation

Genetic transformation of cassava was first achieved by Calderón-Urrea in 1988. Since then several laboratories have improved the protocol for the transformation and/or regeneration of calli (Taylor *et al*., 2004). Transformation has been achieved in a variety of

cassava germplasm and the introduction of several different genes. Traits to be improved range from modification of starches (Zhao *et al.*, 2011) introduction of enhanced protein content or silencing the genes related to the molecular pathway for the production of cyanogenic glucosides. Zhang Peng *et al.*, reported in 2008 on genetic transformation activities conducted in Asia for different traits, relevant to cassava.

5. PERSPECTIVES AND CHALLENGES

During the past 30-40 years, significant progress has been achieved in the initial phase of the scientific genetic improvement of cassava. In a way it could be said that the adaptation of the crop to more intensive cultivation systems has been completed. This process involved assembling major traits, such as improved yield (mainly through a higher harvest index), low cyanogenic content (when desirable), improved plant architecture and resistance/tolerance to the major diseases and pests.

Future activities involve an increasing emphasis on complex traits such as higher yield and dry matter content in the roots, early bulking, etc., which are more difficult to improve. It is critical for cassava that efficient methods for the improvement of these complex traits are found to maintain the competitive edge that this crop currently has in tropical regions as an alternative to imported carbohydrate sources from temperate regions. Several approaches have been taken to address this situation in recent years. Modifications of the breeding scheme have been implemented for a more dynamic recurrent selection system and for obtaining valuable information on the breeding value of parental clones. Biotechnology tools have been adapted to cassava and are currently incorporated in different projects for its genetic improvement. A molecular map has been developed (Fregene *et al.*, 1997; 2000; Mba *et al.*, 2001) and marker-assisted selection is currently used for key traits (Blair *et al*., 2007). Genetic transformation protocols are available and have been used successfully for the incorporation of different genes (Zhang Peng *et al.*, 2008; Taylor *et al.*, 2004). Tissue culture techniques can also benefit cassava through the production of doubled-haploid lines (CIAT, 2008).

As the crop has evolved and new improved varieties that satisfy the most important needs have been released and adopted by farmers, new challenges and opportunities arise. An important need is to introduce herbicide tolerance in the crop. Several approaches can be taken from genetic transformation (Taylor *et al.*, 2004), to screening for the natural occurrence of tolerance to certain herbicides, to the induction of mutations as already demonstrated for different herbicides and different crops (Tan and Bowe, 2008).

The green revolution on wheat (Schmidt, 1984) and rice (Virmani and Ilyas-Ahmed, 2007) was based on changes in the plant architecture taking advantage of the dwarf and semi-dwarf morphology that improved harvest indices. In maize, changes in plant architecture and increased tolerance to high-density planting allowed gradual but drastic increases in plant densities over the last century (Duvick, 1984; Troyer, 2008). **Photo 1** in Chapter 6 on Heterosis and Inbreeding in Cassava (in this publication) illustrates a cassava plant type that could lead to a similar revolution in cassava by drastically increasing plant densities at the farm level, while maintaining productivity per plant relatively unchanged.

One of the challenges for the crop is for a more extensive exploration to increase the germplasm collections and to develop approaches that will allow for an efficient evaluation of such germplasm. In this regard, tools for rapid identification of novel starch types are

needed. The lack of genetic variability for overcoming the problem of post-harvest physiological deterioration remains a major bottleneck for cassava utilization and commercialization, although significant breakthroughs have been achieved recently (CIAT, 2008). The inherent potential of cassava, its capacity to grow in marginal environments and the incorporation of new, powerful biotechnology tools, as described in several of the references provided in this chapter, offer a bright perspective for the crop and the people that depend on it.

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CHAPTER 6

HETEROSIS AND INBREEDING IN CASSAVA: EXPERIMENTAL RESULTS AND PERSPECTIVES

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1. INTRODUCTION

Inbreeding and outbreeding, the mating of genetically like and unlike individuals, respectively, form the foundation of the modern heterosis concept. Early farmers were aware of the significance of inbreeding and outbreeding in plants and animals. Perhaps the earliest example in this regard is the development of a mule (the cross between a donkey and a horse), which exhibits remarkable heterosis for size, strength and endurance (Goldman, 1999). The same author mentions that Indians in the Quezaltenango region of Guatemala and the Hopi Indians in Arizona (USA) made a regular practice of placing seeds of more than one local variety in each hill, with the idea that larger yields could be obtained in this way. This recognition that close planting of different varieties would suggest that the recognition of the benefits of heterosis "was firmly in place many thousands of years ago in the Americas". These ideas were also supported by the widespread acceptance of an incest taboo among many of the world's cultures, indicating that humans have known for thousands of years about the consequences of inbreeding and outbreeding. Heterosis can be described as the increased vigor of growth, survival and fertility of hybrids as compared with the two homozygotes. It usually results from crosses between two genetically different, highly inbred lines. It is always associated with increased heterozygosity and depending of whom the hybrid performance is compared with, there are several ways to measure it (Schegel, 2003).

Early work on the development of a scientific description of heterosis was conducted as early as 1776 when detailed research in tobacco quantified hybrid vigor. Many other scientists made contributions which led Darwin to conduct different experiments where heterosis would be expressed, including maize. He noted the deleterious effects of inbreeding and, interestingly, he also noted that they could be reversed by crossing the inbred strains (Goldman, 1999). Mendel also noted that the hybrids between his tall and short pea varieties were taller than the tall parent. Heterosis can therefore be observed in self-pollinated crops like pea as well as out-crossing crops such as maize. Heterosis, however, is very conspicuous in cross-pollinated crops and it is in that kind of crops (maize, sorghum, sunflower) where most of the work has been done. The foundations of the heterosis concept were built on early works in maize by E.M. East (1909; 1936) and G.H. Shull (1908; 1909). Maize is a crop that shows remarkable levels of heterosis, was a commercially important crop in the USA (when that pioneering work was conducted as well as today) and its reproductive characteristics greatly facilitates inbreeding and outbreeding in a controlled manner. Shull, who coined the word heterosis, published in 1908 and 1909 landmark articles describing the key aspects of inbreeding and outbreeding, inbreeding depression and heterosis. This chapter provides a description of the genetic basis of heterosis along with alternative breeding approaches to exploit it.

Genetic variation for traits can be initially divided into qualitative and quantitative

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based on the number of genes involved, the complexity of the inheritance and the relative importance of the environment in their expression. Mendelian genetics describes simply inherited traits, which are controlled by one or two genes and are not heavily influenced by the environment. Color of the pea flower, for example, was one of the characteristics analyzed by Mendel. Genotypes carrying one or two copies of the alleles for purple flower will always produce purple flowers independently of the environment where they are grown. Qualitative traits, therefore, typically have high heritability values (both, broad or narrow sense heritabilities). Quantitative traits, because of their dependence on the environment, generally speaking, have intermediate to low heritability values.

Basic knowledge about the inheritance of traits is fundamental for efficient and effective crop genetic improvement. Most economically important characteristics are controlled by many genes and strongly affected by the environment (quantitative inheritance). However, little progress has been achieved in understanding the inheritance of traits with agronomic relevance in cassava (Easwari *et al*., 1995; Easwari and Sheela, 1998; Losada, 1990), which rely heavily on heterosis. The cassava situation is unique in that while a molecular map has already been developed (Fregene *et al*., 1997; Mba *et al*., 2001), and many studies of genetic variability among and within landraces have been conducted (Asante and Offei, 2003; Elias *et al.*, 2004; Peroni and Hanazaki, 2002; Sambatti *et al.*, 2001; Zaldivar *et al.*, 2004), little knowledge on quantitative genetics has so far been generated.

Therefore, the cassava-breeding project at CIAT conducted several studies to measure the relative importance of additive and non-additive genetic effects in sub-humid (Cach *et al.*, 2005; 2006), acid soil savannas (Calle *et al.*, 2005; Pérez *et al.*, 2005a) and mid-altitude valleys (Jaramillo *et al.*, 2005; Pérez *et al.*, 2005b) environments. In addition, analyses of inbreeding depression on eight families (Contreras *et al.*, 2008) were also conducted and the most relevant information will be presented in this chapter.

The heterozygous nature of cassava complicates work to improve the existing molecular map and implement marker-assisted selection. Different authors have suggested that cassava is a segmental allo-tetraploid (Umanah and Hartman, 1973; Magoon *et al.*, 1969), which would further increase the complexities of gene interactions within and between loci and between homolog and homeolog genomic components.

Cassava is an interesting crop because its vegetative propagation allows the estimation of within-family genetic variation and, indirectly, the relative importance of epistatic effects. Genetic studies analyzing the importance of epistatic effects are not very common, particularly in annual crops. Accurate measurement of epistatic effects for complex traits, such as yield, is difficult and expensive. Reports in the literature on the relevance of epistasis are not as frequent as those estimating additive and dominance variances or effects, and generally take advantage of the vegetative multiplication that some species offer (Comstock *et al.*, 1958; Foster and Shaw, 1988; Isik *et al.*, 2003; Rönnberg-Wästljung and Gullberg, 1999). In many cases these reports are on forest trees. Because of the complexities of these analyses and the costs involved, reports in the literature related to epistatic effects are frequently based on a limited number of genotypes.

Holland (2001) published a comprehensive review on epistasis and plant breeding. Several cases of significant epistasis have been reported in self- (Gravois, 1994; Pixley and Frey, 1991; Orf *et al.*, 1999) and cross-pollinated (Ceballos *et al.*, 1998; Eta-Ndu and Openshaw, 1999; Lamkey *et al.*, 1995; Wolf and Hallauer, 1997) crops. According to Holland (2001) finding significant epistasis seems to be easier in self- than in cross-pollinated species and in designs based in the contrasts of means, rather than the analysis of variances.

2. GENETIC BASIS OF HETEROSIS

As stated by Crow (1999) almost from the time Medelian inheritance first came to be generally accepted, there have been two alternative theories to explain the phenomenon of heterosis: the overdominance and the dominance hypotheses. The overdominance hypothesis arises from Shull and East's work and is based on the idea that the larger amount of information contained in a heterozygous locus would lead to a phenotype superior to those of both homozygotes. The dominance hypothesis was first proposed by Bruce in 1910 (cited by Crow, 1999). It states that heterosis is the result of the masking of deleterious recessives by dominant or partially dominant alleles, each progenitor bringing to the hybrid a somewhat different collection of favorable dominants. These concepts relate to the gene action models described in the introductory chapter on quantitative genetics (see Chapter 4). There were two main criticisms to the dominance hypothesis: a) the absence of skewed F2 distributions; and b) the failure of selection to produce inbreds as good as the hybrids.

By 1952, during the first Heterosis Conference, the controversy between the two hypotheses explaining heterosis was still unresolved and could be summarized as follows (Crow, 1952): a) The dominance hypothesis can explain the deterioration from inbreeding and the recovery on outcrossing; b) The dominance hypothesis is inadequate to explain how hybrids can greatly exceed the randomly mating populations from which the hybrids were derived; and c) The overdominance hypothesis demands a kind of gene action that is rare, but even if only a small minority of loci are of this type, they may be a major factor in population variance and heterosis. As more experimental data was obtained, the scientific community gradually accepted that the dominance hypothesis better explained field observations. By 1983 G. Sprague wrote:

"Studies have shown that additive and dominance gene effects are generally much greater that other types of gene effects. Additive effects are precisely those which respond to selection. Specially designed experiments have shown that both overdominance and epistasis exist, but neither has been shown to be important at the population level… Thus, as far as the maize breeder is concerned, a pragmatic solution to the dominance-overdominance controversy has been reached. Additive and dominance effects provide a satisfactory model for the heterosis and for the rather remarkable progress achieved through breeding. Genetic variances estimates for populations under selection indicated little decrease in variability, thus giving assurance of further substantial progress."

In 1999 a second conference on heterosis was held in Mexico City. By then, several scientists presented further information supporting the dominance hypothesis. Duvick (1999), for example showed how the yield increases in hybrids was paralleled (but lagging behind) by increases in productivity of inbred lines. This information would tend to disprove the second objection to the dominance hypothesis described above. There is a general consensus now that dominance was the key factor explaining heterosis, but that overdominance is probably present in a few loci, but they must be "a small minority" (Crow, 1999; Troyer, 2006).

The relative importance of epistasis in the expression of heterosis remains unclear. This is not surprising given the difficulties of quantifying the actual effect of epistasis in the expression of different traits. Sprague (1983) suggested that it played a minor role. On the other hand, Goodnight (1999) provided an interesting perspective of the impact of epistasis in the expression of heterosis and suggested that epistasis indeed may play a more important role in the expression of heterosis than previously credited. Troyer (2006) also includes epistasis as an important action in the expression of heterosis in maize. One important idea

contributed in this article was that heterosis has played a major role through increased tolerance to stresses. If that is the case, when so many concerns are arising regarding the consequences of climate change, the relevance of heterosis in the future may be even greater than in the past. An excellent and comprehensive review on heterosis was published by B. Mukherjee, in 1996.

3. ADDITIVITY, DOMINANCE AND EPISTASIS: EXPERIMENTAL RESULTS IN CASSAVA

Three different diallel studies were conducted using three different sets of cassava parents targeting specific environments: sub-humid, acid soil and mid-altitude valleys environments (Cach *et al.,* 2005a; 2005b; Calle *et al.,* 2005; Jaramillo *et al.,* 2005; Pérez *et al.,* 2005a; 2005b). These studies took advantage of the vegetative propagation of cassava that allows separation of the genetic from the environmental variation within family. The within-family analysis allows estimation of the relative importance of epistatic effects (Hallauer and Miranda, 1988)

3.1 Sub-humid environment

The analysis of variance and other details of this study have been published (Cach *et al.,* 2005a; 2005b). Based on the magnitude of the estimates for between- and within-family genetic variances, a large proportion of the genetic variability (79-93%) remained as within family variation (**Table 1**). As expected, the lowest within-family variation (79% of total genetic variance) was measured for a relatively simply inherited trait such as the reaction to thrips (Bellotti, 2002), which showed the only statistically significant additive variance. The tolerance/resistance in outstanding parents transmitted to the progeny tended to accentuate differences among families and reduce the variability among sister clones. However, it is clear that a considerable within-family variation still remained even for the reaction to thrips. On the other hand, complex traits such as root and foliage yields showed a larger partitioning of the total genetic variance (>90%) into the within-family variation, suggesting that there were, comparatively, smaller differences in the breeding values of the progenitors.

Dominance effects were very important for thrips, harvest index, and root and foliage yields, with variance estimates significantly different from zero (estimates two times or more the size of the respective standard error). Only the score for thrips and dry matter content showed larger estimates for the additive compared with the dominance variance (**Table 1**). This highlights the importance of heterosis in cassava breeding for many relevant traits, which in turn justifies the implementation of a reciprocal recurrent selection scheme for cassava genetic improvement. Epistatic effects were significant for all variables, except harvest index, based on the test for epistasis.

3.2 Acid-soil savannas

Results of this diallel have been published (Calle *et al.,* 2005; Pérez *et al.,* 2005b). As in the previous diallel, a large proportion of the genetic variability was detected as within-family variation for fresh root and foliage yields (**Table 2**). The within-family genetic variances for harvest index, dry matter content and plant type score were larger than for between-family variation, but the difference was not as large as for the root and foliage yields. On the other hand, the score for super-elongation disease (SED) induced by the fungus *Sphaceloma manihoticola,* showed larger variation in the between- compared with the within-family component. Larger between-family variation was observed for reactions

to thrips, white flies and mites in the diallels for the sub-humid and mid-altitude valleys environments and with a different set of parental lines (Cach *et al.*, 2005b; Pérez *et al.*, 2005a). The relatively simple inheritance for resistance to diseases or pests (with a strong dominance component) generates large variation between the averages of progenies involving one or two resistant parents compared with those from susceptible ones, with relatively little or no variation among the individual genotypes or clones within each family.

Table 1. Variances and test for epistasis from the evaluation of a diallel set combining data from two locations (Pitalito and Sto. Tomás) in Atlántico Department, Colombia. Within parenthesis the standard error for each estimate is provided.

Genetic parameter	Thrips (1-5)	Fresh root yield	Fresh foliage yield	Harvest Index	Dry matter content	Dry matter yield
σ^2_G (BetweenF_1)	0.225	13.09	11.53	0.0010	0.772	0.694
σ^2_G (Within F_1)	0.641	127.21	131.86	0.0037	5.556	9.977
σ^2_G (Total)	0.867	140.30	143.39	0.0048	6.328	10.671
σ^2_A	0.419	17.82	11.93	0.0009	1.452	0.741
	(0.211)	(13.75)	(12.59)	(0.0010)	(0.985)	(0.933)
σ^2_D	0.231	23.87	27.02	0.0027	0.765	1.589
	(0.068)	(11.15)	(10.00)	(0.0011)	(0.497)	(0.919)
Epistasis test[1)]	0.259	100.40	105.64	0.0013	4.257	8.414
	(0.119)	(12.74)	(11.84)	(0.0009)	(0.673)	(0.990)

[1)] Test for epistasis = $\sigma^2_{c/F1}$ – 3 Cov. FS + 4 Cov. HS

The cassava-breeding project at CIAT has recently started to generate data from the earlier phases of the selection process that allow an estimation of the breeding value of parents used in generating these trials. In general, these estimations of breeding values based on the CET will be effective in traits were the genetic variation is concentrated in the between-family component or shows strong additive effects. Selection of outstanding parents for a given trait such as SED score, will tend to generate uniform progenies also outstanding for that trait. This, in turn, could allow the implementation of the Backward GCA Selection described by Mullin and Park in 1992. For characteristics such as fresh root yield, with strong non-additive effects and large within-family variation, the selection of outstanding parents would not be enough and individual clone analysis, within a given family, would be required. In this environment, epistatic effects were important only for fresh root and foliage yields. These results agree with those observed in similar studies conducted for the other environments.

3.3 Mid altitude valleys

Results from this diallel have been published (Jaramillo *et al.*, 2005; Pérez *et al.*, 2005a). In this diallel, as in the previous ones, a large proportion of the genetic variability was found to be within-family variation (**Table 3**). It was surprising to find such a large variation for the within-family component, for reaction to the two pests (mites and white flies), which was mostly attributable to additive variation.

Table 2. **Variances and test for epistasis from the evaluation of a diallel set from ten parents**

combining data from two different edaphic environments at CORPOICA-La Libertad (Villavicencio) in Meta Department, Colombia. Within parenthesis the standard error for each parameter is provided.

Genetic parameter	Fresh root yield	Fresh foliage yield	Harvest Index	Dry matter content	Plant type score	SED[2)] score
σ^2_G (Between F_1)	1.649 (2.954)	1.325 (3.094)	0.0010 (0.0006)	1.600 (0.664)	0.089 (0.039)	0.237 (0.055)
σ^2_G (Within F_1)	21.082 (2.297)	38.557 (3.242)	0.0030 (0.0003)	3.216 (0.169)	0.121 (0.012)	0.088 (0.066)
σ^2_G (Total)	-1.485 (6.321)	1.172 (8.035)	0.0015 (0.0016)	3.379 (2.399)	0.160 (0.144)	0.523 (0.234)
σ^2_A	9.028 (7.930)	3.384 (6.594)	0.0011 (0.0013)	0.873 (0.666)	0.096 (0.033)	0.092 (0.050)
σ^2_D	15.054 (6.740)	35.433 (6.858)	0.0014 (0.0012)	0.872 (1.294)	-0.031 (0.077)	-0.242 (0.139)
Epistasis test[1)]	1.649 (2.954)	1.325 (3.094)	0.0010 (0.0006)	1.600 (0.664)	0.089 (0.039)	0.237 (0.055)

[1)] Test for epistasis = $\sigma^2_{c/F1}$ – 3 Cov. FS + 4 Cov. HS

[2)] SED = super-elongation disease induced by the fungus *Sphaceloma manihoticola*

Table 3. Variances and test for epistasis from the evaluation of a diallel set from nine parents combining data from two different mid-altitude valleys environments at CIAT-Palmira and Jamundí in Valle del Cauca Department, Colombia. Within parenthesis the standard error for each parameter is provided.

Genetic parameter	Fresh root yield	Harvest Index	Dry matter content	Reaction to mites	Reaction to whiteflies
σ^2_G (Between F_1)	42.8 (13.3)	0.0016 (0.0004)	1.19 (0.43)	0.271 (0.067)	0.345 (0.115)
σ^2_G (Within F_1)	288.9 (19.2)	0.0029 (0.0002)	2.25 (0.21)	0.188 (0.107)	0.119 (0.120)
σ^2_A	11.9 (24.7)	0.0029 (0.0015)	1.43 (1.33)	0.571 (0.271)	0.994 (0.467)
σ^2_D	152.1 (49.1)	0.0018 (0.0008)	2.47 (0.89)	0.170 (0.065)	-0.210 (0.132)
Epistasis test[1)]	168.9 (40.2)	0.0001 (0.0010)	-0.32 (0.92)	-0.225 (0.179)	-0.221 (0.279)

[1)] Test for epistasis = $\sigma^2_{c/F1}$ – 3 Cov. FS + 4 Cov. HS

The magnitude and generalized significance of σ^2_D highlights the importance of non-additive genetic effects (heterosis) in this allogamous species. Only the reactions to pests showed significant estimates for σ^2_A, not only in this diallel but in the other two as well. In this third diallel study, fresh-root yield was the only trait showing significant conditioning by epistatic effects, following the same trend observed in the previous studies.

Attempts to quantify epistatic effects frequently fail to reach statistical significance, in part, because of the size of the standard errors typical for complex linear functions (Hallauer and Miranda, 1988; Holland, 2001). In these diallel studies, however, this was not the case and epistasis was consistently important for complex traits such as fresh root yield.

One major constraint for the introduction of inbreeding in cassava is the time required for it. The production of doubled haploids through anther or microspore culture is an interesting approach that would reduce the time required to obtain homozygous genotypes. This, in turn, will maximize the exploitation of dominance and epistatic genetic variation, which have been found to be significant in this study.

4. INBREEDING DEPRESSION IN CASSAVA

The phenotypic mass selection used for cassava breeding takes advantage of the vegetative propagation of the crop (Morante *et al.*, 2005). In selecting outstanding clones, all genetic effects (additive, dominance, and epistatic) are exploited (Hershey, 1984; Jennings and Iglesias, 2002). However, the current recurrent selection system lacks the capacity to direct genetic improvement in such a way that the frequency of favorable genetic combinations (within or between loci) is maximized. Results presented above provide an idea about the relative importance of additive, dominance and epistatic effects on the inheritance of several traits, which have economic relevance for cassava. To achieve this, special efforts to design parental clones that produce better crosses are required. The development of clones, specifically designed for their use as parents in breeding nurseries, would be one alternative that offers interesting advantages.

Introduction of inbreeding in cassava genetic enhancement offers several advantages (Ceballos *et al.*, 2004; Ceballos *et al.*, 2007a). It would facilitate the gradual and consistent assembly of favorable gene combinations, which in the current system occurs just by chance. Inbreeding would also facilitate the reduction of the genetic load of this crop, which is expected to be relatively large at this stage of the evolution of the crop. Inbreeding was involved in the identification of natural (Ceballos *et al.*, 2007b) or induced mutations (Ceballos *et al.*, 2008), illustrating its relevance for discovering commercially useful recessive mutants. Other advantages of homozygous progenitors in the cassava-breeding project include the possibility of implementing the back-cross scheme, facilitated germplasm exchange and conservation (as botanical seeds that breed true) and cleaning disease-contaminated planting material of elite hybrids (remaking the hybrid by crossing again the original progenitors).

As convincing as these arguments are to justify the introduction of inbreeding in cassava there are several issues that need to be addressed. Reaching homozygosity through successive self-pollinations may require as many as 12 years. As an alternative strategy, significant progress has been achieved in developing a protocol for the production of doubled-haploids through microspore isolation and culture (CIAT, 2008). Analysis of genetic variability in cassava revealed that it is a highly heterozygous species. Cassava, being an outcrossed crop, abhors inbreeding and is expected to show severe depression both in traditional (Pujol *et al.*, 2005) and modern production systems (Kawano *et al.*, 1978). As was the case of temperate maize in the early 1900s and tropical maize by the 1970s, the crop will therefore need to be improved for its tolerance to inbreeding depression. A few recurrent selection cycles (self pollinating each elite clone down to the S_2 level, and recombining the surviving progenies) should help prepare the materials for the trauma of total homozygosity. However, there is a widely held concern that inbreeding depression could be too severe in cassava to allow reaching true homozygosity in a plant that is still

viable and able to bear seeds for the generation of the hybrid genotypes.

CIAT therefore conducted this study to quantify inbreeding depression for different traits in eight S_1 families; and to analyze the variation within these eight groups of S_1 cassava families.

4.1 Materials and methods

Self-pollinations were obtained with each of eight elite clones and the seed was germinated under greenhouse conditions. At harvest time S_1 progenies from eight progenitors were chosen based on the size (number of segregating progenies) of the respective S_1 families. Only families that had at least 100 S_1 genotypes were selected. Within family selection of particular genotypes was avoided, the only criterion being the capacity to produce enough planting material to clone each genotype, which is expected to have little influence on the genetic make up of each family.

From each S_1 plant, 11 cuttings were obtained. Nine of these cuttings were used to plant a field experiment based on three replications with three plants each. Evaluations were conducted in a single location, using a complete block experimental design. Progenies from each parental genotype were evaluated in separate experiments, for a total of eight different and independent trials. The remaining two stakes were used to maintain each genotype for further studies. Every 10-12 plots, a plot (also with three plants) with the parental clone of the respective S_1 family, was planted to serve as the S_0 check. Planting distances were the standard 1x1m for a total plant density of 10,000 plants/ha. Experimental plots were surrounded by two rows planted with the respective S_0 checks.

Variables analyzed were plant height (cm), measured from the soil level to the highest apical point of the plant at harvest time; fresh root yield (kg/plant); harvest index (root biomass as proportion of total biomass); dry matter content (measured as %) was determined by the specific gravity methodology as suggested by Kawano *et al.* (1987). Inbreeding depression (ID) was estimated for each variable as a percentage of the S_0 average:

$ID = [(S_0 \text{ mean} - S_1 \text{ mean})/ S_0 \text{ mean}]*100.$

Therefore, the lower the ID value, the lower the depression, which implies that the performance of the S_1 progenies is close to that of the S_0 progenitor.

4.2 Quantification of inbreeding depression in cassava

Table 4 presents a summary of the results for the eight S_1 families evaluated. The average performance of the elite progenitors (inter-planted every 10-12 plots) is provided as well as the ID value for each family and trait. For plant height, average ID was about 10%, meaning that on average the height of S_1 genotypes was as much as 90% of that of the S_0 progenitors. The range of ID among the different S_1 families ranged from 0.7 (AM 334) up to 24.0% (AM 339).

ID for fresh root yield was, as expected, considerably higher with an average of almost 64%. In other words, S_1 genotypes yielded only 36% of the yield achieved by their respective progenitors. The highest ID was observed in family AM 320 with ID= 77.8% and the lowest value was found in AM 337 with ID = 50.6%. Average IDs for other traits were 37.9% for fresh foliage production (ranging from 16.4 to 56.5%), 26.5% for harvest index (ranging from 16.6 to 43.0%) and 5.3% for dry matter content (ranging from 0.3 to 8.7%). Family AM 320 showed the highest levels of ID for fresh root yield, fresh foliage yield and dry matter content and had the second highest ID for the remaining variables (plant height and harvest index).

Table 4. **Inbreeding depression (ID) as % of the performance from the S_0 generation measured in eight S_1 cassava families.**

Family	Plant height (cm)		Root yield (kg/plant)		Foliage yield (kg/plant)		Harvest Index (0-1)		DMC (%)	
	S_0	ID	S_0	ID	S_0	ID	S_0	ID	S_0	ID
AM 320	203	15.6	4.48	77.8	2.51	56.5	0.62	38.5	30.0	8.7
AM 331	246	6.9	9.29	65.8	2.94	27.0	0.76	25.2	29.7	6.9
AM334	224	0.7	4.92	56.9	2.57	31.9	0.65	18.0	26.1	1.5
AM335	217	10.6	4.50	64.0	1.80	42.8	0.71	16.6	35.3	8.7
AM336	208	9.6	1.03	61.7	1.96	33.1	0.33	43.0	29.7	0.3
AM337	175	6.0	3.29	50.6	1.93	16.4	0.63	25.2	32.1	2.9
AM338	208	7.6	4.23	65.6	2.70	50.9	0.61	20.2	31.8	4.5
AM339	239	24.0	3.52	68.8	1.86	44.5	0.65	25.3	35.8	7.0
Average	**215**	**10.1**	**4.40**	**63.9**	**2.30**	**37.9**	**0.62**	**26.5**	**31.3**	**5.3**

[1)] DMC = dry matter content

In addition to the average values of ID, the range of ID for each family is also provided in **Table 5.** For plant height, only family AM 339 failed to produce S_1 genotypes with plant height superior to that of the S_0 progenitor genotype. In contrast, IDs observed in family AM 334 ranged from 28.5 to -34.1. The average maximum ID observed for plant height was 38.2 and the minimum ID averaged -19.5 (a plant height almost 20% above that of the progenitor genotype). For root yield, figures were different. The maximum levels of observed ID averaged 95.8% (suggesting an almost negligible production). The minimum ID for root yield showed, in many cases, negative values (yields higher than in the progenitor genotype). Only families AM 331 and AM 339 failed to produce S_1 genotypes with fresh root yield above those observed in their progenitor. The average minimum ID values suggest that on average across the eight families analyzed, the best clones yielded 23% higher than their respective progenitor clone. For fresh foliage yield and dry matter content every family showed S_1 genotypes with values above their progenitor clone. In the case of harvest index, however, families AM 320 and AM 331 failed to produce S_1 genotypes with a performance superior to their respective progenitor clone. Additional results from this study have already been published (Contreras *et al.,* 2008).

4.3 Implications of the measured levels of inbreeding depression in cassava

The way this study was conducted would tend to overestimate the significance of ID compared with similar studies conducted on maize. When this parameter was evaluated in maize, typically S_0 populations were randomly self-pollinated to generate a representative S_1 generation, which was then evaluated for comparison. Representative samples of the S_0 and S_1 populations were therefore compared. In cassava, however, individual elite genotypes represented the S_0 generation, not a population. It can be visualized that the eight elite clones used as progenitors were actually the best genotypes (not the averages) out of their respective S_0 generation. They are not, therefore, a representative sample of the average performance at the S_0 generation as it is usually done in the case of maize. On the other hand, the unavoidable selection of genotypes that had the capacity to produce enough stakes (so individual genotypes could be cloned) may have resulted in some underestimation of ID. This selection, however, is closely related to plant height, which showed the second lowest level of depression (10.1%). Therefore the actual effect of selecting plants that could

produce enough planting material was most likely, negligible.

Table 5. Ranges for inbreeding depression (as % of the performance from the S_0 generation) measured in eight S_1 cassava families.

Family	Plant height (cm)		Root yield (kg/plant)		Foliage yield (kg/plant)		Harvest Index (0-1)		DMC (%)	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
AM 320	48.2	-15.1	100	-48.2	94.0	-27.8	100	27.9	33.1	-20.1
AM 331	29.6	-23.2	95.1	13.8	83.5	-79.3	54.3	2.6	31.3	-13.1
AM334	28.5	-34.1	91.9	-17.2	80.6	-49.0	47.6	-18.8	26.8	-18.3
AM335	30.8	-17.6	93.7	-13.2	89.8	-26.8	67.5	-16.6	29.7	-6.5
AM336	54.3	-29.9	100	-52.6	89.8	-60.1	100	-68.0	21.0	-25.7
AM337	35.2	-17.3	94.3	-60.9	72.4	-68.2	82.3	-14.7	28.7	-28.6
AM338	37.4	-22.8	94.5	-27.8	82.3	-23.5	75.8	-22.1	19.4	-5.8
AM339	41.5	3.9	97.2	21.0	89.2	-13.0	71.1	-17.7	21.4	-3.6
Average	**38.2**	**-19.5**	**95.8**	**-23.1**	**85.2**	**-43.5**	**74.8**	**-15.9**	**26.4**	**-15.2**

[1)] DMC = dry matter content

Previous studies of inbreeding depression in cassava and maize

In tropical maize, García and co-workers (2004) analyzed ID in S_1 families from six different populations. From each of these populations, two cycles of recurrent selections were used as the S_0 generation. ID, therefore, could be quantified at two different phases of the evolution in these six basic populations. Average ID across the 12 S_0-S_1 comparisons was around 50% for grain yield (ranging from 37.9 to 67.2%). Inbreeding depression was higher in advanced cycles of recurrent selection (52.2%) compared with the initial cycles (48.9%). For plant height, average ID was around 13% (ranging from 7.1 to 21.6%) and it tended to be slightly higher (13.4%) in advanced cycles of recurrent selection than in earlier ones (12.2%). Pacheco and coworkers (2002) made the comparison between S_0 and S_1 families from 28 subtropical maize populations, evaluated across ten environments. Average ID for grain yield was 49% and ranged from 34.6 to 59.2%. As expected, the highest ID was measured in populations that had never been exposed to inbreeding. Miranda Filho (1999) reported average (five different maize subtropical populations) ID values for grain yield and for plant height (three populations) of 44.9% and 8.7%, respectively.

In temperate maize, Lamkey and Smith reported in 1987 average ID in 11 populations of 27% (ranging from 22.7 to 44.4%). This study included populations from seven different eras of maize breeding. Another study on temperate maize, involving four different types of S_0 populations and their S_1 derivatives (Walters *et al.,* 1991), found average ID for grain yield and plant height of 24.5% (ranging from 18.2 to 34.5%) and 8.6% (ranging from 8.1 to 9.8%), respectively.

In an earlier and pioneering work, Kawano and co-workers (1978) conducted a study similar to the one reported here. S_1 clones from 12 genotypes were produced and evaluated. However, on average, only 16 S_1 genotypes were derived from each parental S_0 clone (ranging from 5 to 36). In the Kawano study, average ID for fresh root yield was 51.8% whereas, in this study, it was 63.9%. Range of variation for ID was wider in the earlier work (-22.6 to 87.5%) compared with the current study (51-78%). The wide variation for ID in Kawano's work (including one case where a set of S_1 genotypes yielded, on average, 22.6% more than the S_0 progenitor) may be the result of the small samples of segregating S_1 genotypes used in that study.

Understanding inbreeding depression in cassava

One of the main concerns regarding the introduction of inbreeding in cassava genetic enhancement has been the valid fear that ID would be too severe to be a practical and feasible approach. Compared with ID in tropical and subtropical maize, which is about 50%, the values found in this study are not unacceptably high. It must be emphasized that comparisons between maize and cassava should be made with caution because of our suspected over-estimation of ID (the S_0 reference point not being the average of the non-inbred population, but a selected elite genotype). It can therefore be concluded that there is no evidence that ID in cassava is unacceptably high and, therefore, it is valid to think about the possibility of using fully homozygous progenitors in future genetic enhancement of cassava.

Taking the ID values from Kawano's work with caution (due to the small number of S_1 genotypes), there is an apparent increase of ID from that study to the current one. This situation is similar, to some extent, to the observations made on maize by García *et al.*(2004), where breeding accentuated ID (from and average of 48.9% to 52.2%). It should be pointed out that no inbreeding was involved in the evolution from earlier to later cycles of recurrent selection (which was based on full-sib families) in García's report. If the progenitors used in Kawano's and the current study are considered representative of two eras of cassava breeding, the increased ID values observed in the current study could be explained as a result of improved functional heterosis in the current progenitors. There is a strong association between heterosis and ID as they are opposite ends of basically the same phenomena (Lamkey and Edwards, 1999; Miranda Filho, 1999).

Implications for breeding

In general, ID in temperate maize was much lower than that of tropical and subtropical maize, with an average of 27%. However, the older population used in the Lamkey and Smith (1999) study (BSSSCO), which had been developed in the early 1930s, exposed an ID of 44%. The contrast between temperate and tropical/subtropical maize is probably due to the fact that early work in temperate maize quickly reduced genetic load in the populations and increased tolerance to ID (from the 44% seen in BSSCO to the levels observed for maize from later eras, with the average values of around 27%). The key element is that recurrent selection in maize included some degree of inbreeding, which quickly built up tolerance to ID. Therefore, it is expected that ID values observed for cassava in the current study (64%) should be reduced drastically, particularly in the first few cycles of recurrent selection, provided that recurrent selection involving some degree of inbreeding is utilized. This expectation is precisely one of the reasons for introducing inbreeding in cassava genetic enhancement for the rapid elimination of deleterious factors that currently represent a large genetic load for the breeding populations of the crop.

Inbreeding depression for plant height in cassava was much lower (around 10%) than for fresh root yield. This value was similar to those observed for maize and smaller than the one measured in Kawano's study (16.7%). ID for harvest index was 26.5%, a much higher value than that reported in Kawano's work (11.0%). Since harvest index is influenced by fresh root yields and plant height, this increase observed for harvest index is probably due to the higher ID values for fresh root yield.

Tolerance to inbreeding can be improved. Very early in maize research it was already suggested that this was possible as implied by the following citation from Eugene Davenport (cited by Goldman, 1999):

"The effect of inbreeding appears both pronounced and disastrous; the second generation from inbred seed being less than two-thirds normal size and nearly barren… but the second planting from this seed when closely selected after the same plan left almost a full stand, which shows that corn may be brought much nearer a constant type than has ever yet been done"

This historic statement is very important because it demonstrates again that, early on, inbreeding depression in maize was very severe (as it currently is in cassava), but that it can be quickly overcome by simple selection procedures. As reported by Duvick (1999), by 1930 inbred maize lines productivity averaged 2 t/ha. Fifty year later, average productivity of inbred lines was above 4 t/ha. Therefore, there are sound reasons to justify the expectation that tolerance to inbreeding depression in cassava can be build up through different recurrent selection approaches.

Inbreeding depression and genetic variances for relevant traits in cassava

Cassava has been demonstrated to be a highly heterozygous species (Kawano *et al.*, 1978; Pujol *et al.*, 2005). Inbreeding depression such as that observed in the current study was, therefore, to be expected. ID is the result of a reduction of the heterozygosity levels in loci with dominance gene effects and because of the increased frequency of expression of unfavorable alleles (Falconer, 1989; Miranda Filho, 1999; Vencovsky and Barriga, 1992). Higher ID is to be expected in materials with high levels of heterozygosity approaching allele frequencies around 0.5. There is a close association between the ID values observed in this study and the relative importance of additive and non-additive effects (including epistasis) reported in three different sets of diallel studies (Cach *et al.*, 2006; Perez *et al.*, 2005a; 2005b). The higher the relevance of non-additive effects in the diallel studies, the higher the ID for the different variables in the present study. Dry matter content had negligible non-additive effects across the three diallel studies, and showed the lowest ID in the current study (5.3%). On the other hand, in the case of fresh root yield, non-additive effects were 6.5 times larger than additive effects (across the three diallel studies) and showed the highest ID in the current study (63.9%). Non-additive effects were 2.3 times larger than additive effects for fresh foliage yield, a variable that expressed considerably lower levels of ID (37.9%) compared with fresh root yield. Finally, for the last variable that can be compared (harvest index), it showed relatively low levels of ID (26.5%) and additive and non-additive effects had about equal relevance in the diallel studies.

The kind of information provided in **Tables 4** and **5** has not been considered in studies of ID in maize. In the current study, ID of individual S_1 segregating clones (genotypes) measured and their frequency distributions could be analyzed. Very relevant is the fact that, even for fresh root yield, in most families few S_1 individual genotypes could be found with yields similar or above the parental S_0 progenitor. High magnitude skewness (either positive or negative) in the distribution of frequencies would suggest that dominance effects are relevant in the inheritance of the trait. A low skewness describes a more symmetrical distribution of frequencies, which are more typically related to additive effects. The degree of skewness observed in the segregating progenies (Contreras *et al.*, 2008) also correlated well with data from the diallel studies and with the criterion described above. The highest skewness was observed for fresh root yield (1.30), intermediate values were observed for fresh foliage yield (0.68), and low values for harvest index (-0.26) and dry matter content (-0.19).

The variations for ID within each of the eight S_1 populations will be further

analyzed and correlated with molecular markers that have been obtained from each genotype in several of these families. In general, variation in ID from one family to the next will depend on the frequencies of alleles relevant for the trait as well as the genetic load in the progenitor clones.

5. JUSTIFICATION AND ADVANTAGES OF INBREEDING IN CASSAVA

As already explained above, cassava is a highly heterozygous species and as such shows strong inbreeding depression for several traits, but particularly fresh root yield, which showed a depression of around 64%. However, inbreeding depression for plant height (a trait related to plant vigor) was considerably lower, around 10%. This is important because it would suggest that there should not be major biological limitations for homozygous cassava plants to produce viable plants that can flower, and bear at least a few viable botanical seeds. These findings are also important because for fresh root yield to show such strong levels of ID, would also suggest a huge opportunity for exploiting heterosis, which is the opposite phenomenon to inbreeding depression. The introduction of inbreeding in the process of cassava breeding offers several advantages that are summarized below.

5.1 Reduction of genetic load

The heterozygous nature of cassava allows for a large frequency of undesirable alleles to be maintained in breeding populations taking advantage of their frequent recessive nature. All the undesirable and deleterious alleles present in a given individual is known as genetic load. Inbreeding exposes these undesirable alleles, allowing for the elimination of genotypes exposing them. The ultimate consequence of this action is that there is a gradual reduction of the frequencies of these undesirable alleles. The evolution of productivity in maize inbred lines in the past century is a perfect example of the impact that a reduction of genetic load can have in a given crop (Duvick, 1999). The introduction of inbreeding, to produce fully homozygous or partially inbred progenitors, would allow for a rapid reduction in the levels of genetic load in elite cassava germplasm and should lead, by default, to improved performance of the hybrids they produce.

5.2 Discovery of useful recessive traits

This is opposite to the argument presented above. It is recognized that some recessive traits may be desirable. Examples have already been reported for many crops in the literature and are gradually emerging for cassava as well. Ceballos *et al.* (2007b; 2008) reported on two recessive starch mutations that were found through self-pollinating accessions of the germplasm bank and through mutagenized populations, respectively. Routine production of inbred germplasm would allow for the identification and subsequent exploitation of these useful recessive traits. As another example, **Photo 1**[3] illustrates a peculiar plant type that was recently identified in an S1 family (obtained after self-pollinating an accession from the germplasm collection at CIAT). A total of 12 plants were grown and half of them showed the phenotype depicted in **Photo 1.** Leaves lacked petioles and in several cases there was no flowering or branching. This particular phenotype could be interesting because the foliage, if harvested, would be of much better quality (petioles contribute considerably to fiber of dried foliage flour, limiting its uses in animal feeding). More importantly, this particular phenotype could allow for higher plant densities (perhaps as high as 30,000 or 40,000 plants per hectare). As explained above, one of the reasons for

[3] For color photos see page 729.

the increased productivity in maize has been higher plant densities (Duvick, 1999). Although there is a lot to learn about this recessive trait and perhaps its usefulness will never materialize, it serves as an example of the potential benefits of inbreeding.

5.3 Possibility of implementing the back-cross scheme

The research conducted in cassava during the past 20-30 years is finally producing a large volume of useful information and the identification of useful germplasm. Many examples of sources of resistance to diseases (Hahn *et al.,* 1980a; 1980b) and pests (Bellotti *et al.,* 2002), or desirable root quality traits (Ceballos *et al.,* 2007b; 2008) have been identified. However, the impact of these high-value characteristics is limited because their introgression requires breeding a new variety *de novo*. For example, Thailand was the first country to invest in the development of a commercial waxy (amylose-free) starch cassava variety. The process implies making crosses between the source of waxy starch with elite germplasm to produce F1 genotypes which will not produce waxy starch given the recessive nature of the waxy mutation. Several unrelated F1 genotypes will then be crossed to produce an F2 generation that will show a certain proportion of progenies with waxy starch. However, if the parents of an elite clone (such as the widely grown cultivar KU50) were homozygous, the introgression of the waxy starch trait would be straight through a back cross scheme. In a few cycles a waxy version of the two parents could be available and when crossed they would produce exactly the same outstanding KU50 hybrid, but with the waxy starch trait expressing. In other words, there would be no need to develop again such an outstanding hybrid. Unfortunately, all cassava breeding projects use heterozygous progenitors preventing the application of the back-cross scheme, which is one of the most widely used and successful breeding approaches used both in self- and cross-pollinated crops (Allard, 1960). Similarly, assuming that whiteflies become an unmanageable problem in a given country, the dominant source of resistance found in MEcu 72 (Bellotti, 2002) could be crossed with one (or two) of the parents of the most outstanding hybrid grown in that country. Provided they were homozygous they could be recovered completely (with the exception of the introgressed source of resistance to whiteflies) through the backcross scheme. These progenitors would have been '*converted*' to being resistant and when crossed the outstanding hybrid they produce would carry now the source of resistance to the insect. So, by a very controlled and predictable way the progenitors and the outstanding hybrid they produce could be turned into one resistant to the white flies. The value of these sources of resistance or high-quality roots increases considerably because their exploitation becomes much more efficient. **Figure 1** illustrates the typical scheme used to introduce a dominant gene into a homozygous progenitor.

5.4 Facilitated germplasm exchange and conservation

The imposition to exchange germplasm *in vitro* (for phytosanitary reasons) restricts considerably the exchange of germplasm between the few cassava-breeding projects of the world. Maintenance of germplasm can only be made through expensive *in vitro* operations or by growing the accession in the field. Both alternatives are expensive and prone to problems that ultimately lead to the risk of loosing germplasm. Since our current effort is directed to identify (by chance) outstanding hybrids the key germplasm to exchange is the finished product (the outstanding hybrid), which generally has only limited application outside the environment where it was developed.

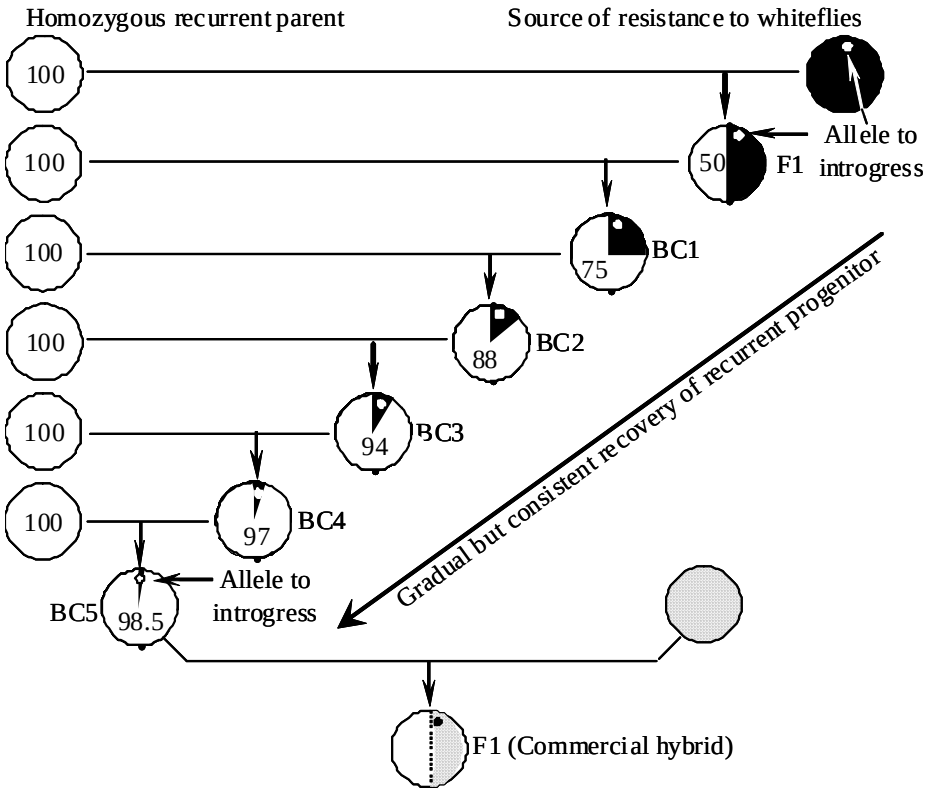

Figure 1. Illustration of the back-cross scheme to introgress a desirable gene into the homozygous recurrent parent through successive back-crosses. There is a gradual recovery of the 'blood' of the recurrent parent. In different stages of the process many progenies are produced but only a genotype that carries the desirable gene is back-crossed to the recurrent parent. Since the progenitor is homozygous each back-cross contributes with gametes that are genetically identical and this allows a gradual and consistent recovery of the recurrent parent. The ultimate objective is to use the 'converted' recurrent progenitor in a cross to produce the same outstanding hybrid but with the addition of the allele that has been introgressed. When the allele to introgress is recessive, self-pollinations need to be made at each back-cross stage to identify which genotypes carry the allele.

If cassava breeding were based on the development of good homozygous progenitors that produce outstanding hybrids, the key research product would not be the hybrid but the homozygous progenitors. Progenitors that are selected because of their adequate general combining ability and breeding value could be shared among breeding projects and crossed with local (homozygous) progenitors in search of outstanding hybrids. That is basically the way the maize breeding industry developed based on a few university projects, which then opened the possibility for the private sector investments. For decades, public and private breeding projects worked and collaborated, exchanging germplasm and information. This process led to the identification of '*venerable*' maize homozygous lines, such as Mo17 and B73. It is impossible to quantify the significance and impact of this synergism, but most likely has played a very significant role in the development of conventional breeding projects in different crops.

5.5 Facilitated phytosanitary maintenance of superior clones

When an outstanding cassava hybrid is identified, it is multiplied and maintained vegetatively. However, the continuous growing of a clone in the field, year after year, eventually results in decreased performance due to the 'contamination' with organisms that can be pathogenic or just epiphytes that do not induce disease proper but ultimately affect the performance of cassava. When disease problems are chronic and severe (e.g. bacterial blight or cassava mosaic disease), the only way to recover the productivity of the clone is by meristem culture to clean the planting material from undesirable micro-organisms. This is an expensive process that could be avoided if the outstanding clones were produced from inbred progenitors. They could be crossed again and the same hybrid would be produced through botanical seed. A few crosses (for example to produce 100-200 botanical seeds) every now and then could provide a new generation of the same hybrid but free of diseases.

5.6 Facilitated conventional and molecular genetic studies

The availability of homozygous progenitors would facilitate greatly the logistics of genetic studies (both conventional and molecular genetics). Segregating progenies could be selected for a higher contrast and 'cleaner' segregations. This, in addition to the obvious fact that a larger number of recessive traits (desirable and undesirable) would be properly identified allowing for the analysis of genetic segregations that we are not aware they are actually happening but remain masked behind the heterozygous nature of the crop.

5.7 Development of superior hybrids by design, not by trial and error

In Chapter 4 describing the basics of quantitative genetics there is a clear illustration (Figure 4) of the way dominance and epistatic effects can be systematically exploited for enhanced heterosis (hybrid vigor). These sources of genetic variation can also be exploited through reciprocal recurrent selection methods (Hallauer and Miranda, 1988) without the use of inbreeding, but genetic gains would be slow. In the case of cassava, as for other crops, the shaping of two reciprocal populations for enhanced heterosis would be very slow if no inbreeding were employed. The development of hybrids in the maize industry can be used as an example of the power that inbred progenitors have in the development of a crop. **Figure 2** presents the evolution of maize yields in the last 150 years (Troyer, 2006).

About 50 to 60% of the gains depicted in **Figure 2** have been demonstrated to be due to genetic causes. The remaining 40 to 50% of the gains are due to management practices such as increases in nitrogen fertilizers and higher plant densities. It has been estimated that 15% of the gains in productivity are due to heterosis (Duvick, 1999).

The sharp increase in maize productivity observed after the year 1935 is due to the shift from farmers planting seed from open-pollinated varieties to planting seed from hybrids produced by crossing selected inbred progenitors. Because the inbred parents available early on still had considerable amounts of genetic load their productivity was low. As tolerance to inbreeding was built, the productivity of inbred lines increased from 2 to 4 t/ha (average of selected elite inbred lines from different eras of maize breeding) allowing the commercial exploitation of single-cross hybrids, which further increased (at a higher rate) grain yields (Duvick, 1999; Troyer, 2006). All data from **Figure 2** depict yields of hybrid maize. Open pollinated varieties were obtained from heterozygous progenitors and were a mixture of hybrids. Double and single-cross hybrids, on the other hand, were derived from inbred parents and only the best performing hybrid (not a mixture) was planted by farmers.

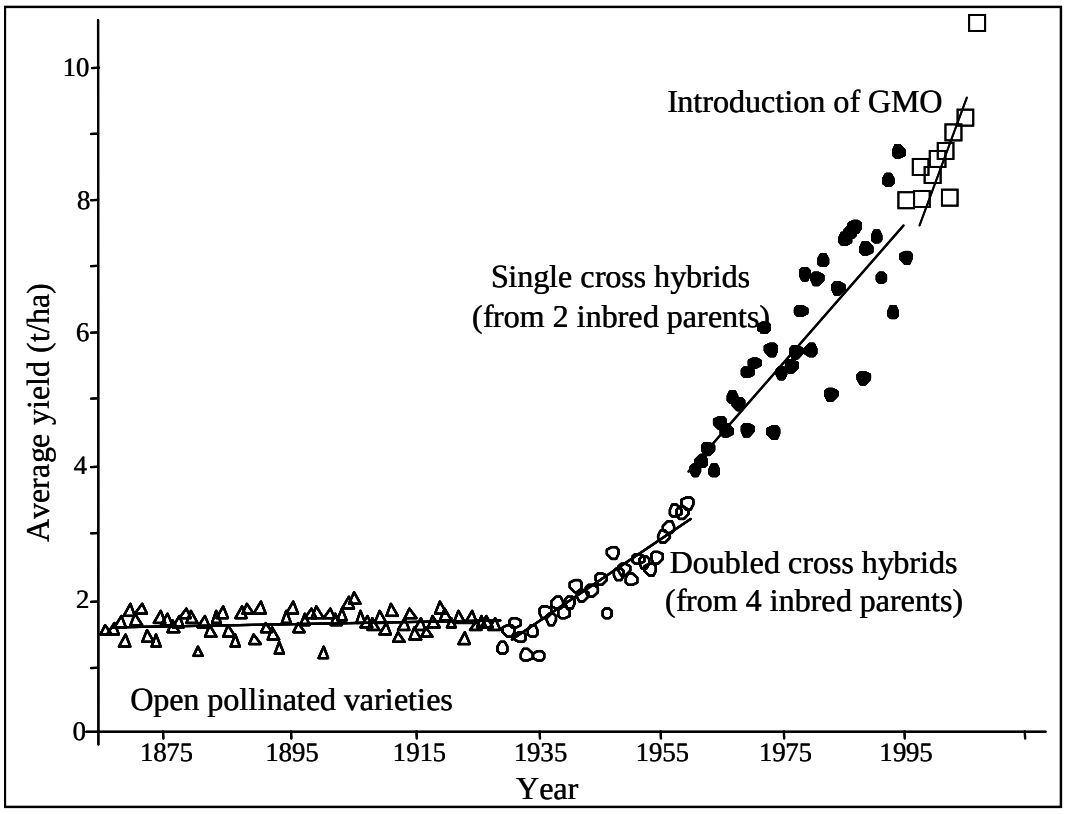

Figure 2. Evolution of maize productivity in the USA during the last 150 years. The introduction of double-cross and single-cross hybrids from inbred progenitors significantly increased maize productivity.
Source: *Adapted from Troyer, 2006.*

5.8 Shortening the length of evaluation and selection cycles

In the current selection approach, crosses among elite (heterozygous) progenitors are made to produce full-sib families. Each seed will represent a unique genotype. The seeds are germinated to produce an F1 plant. Selection can or cannot be made at this single-plant stage. The main function of these F1 plants, however, is to produce vegetative cuttings (7-10 cuttings per genotype) to plant the clonal evaluation trial (CET), which for some breeding projects is the first stage of selection. The seven to ten plants used in the CET, in turn are the source of cuttings for the third stage in the selection process: the preliminary yield trial (PYT), which in the case of CIAT would be based on three replications of 10-plant plots, for a total of 30 plants per genotype. If inbred lines were used to make the F1 crosses, several pollinations between the same inbred parents would yield the same F1 hybrid (as it does in maize). Therefore, by making several crosses among two inbred parents as many as 30 botanical seeds could be produced, in such a way that the first stage of selection would be the PYT. This is very important because: a) it makes the breeding cycle two years shorter by eliminating the F1 and CET stages of selection; and b) it allows the avoiding of these early selection stages, which are based on unreplicated observations and, therefore, prone to large

experimental errors. A better understanding of this advantage can be obtained by reviewing the chapter on Cassava Breeding in this publication.

6. PROBLEMS AND BOTTLENECKS FOR INBREEDING CASSAVA

There are few technical approaches to develop homozygous genotypes. The most common approach has been through successive self-pollinations, which is the fastest method of inbreeding through sexual reproduction. However, in practice this would be impractical because of the time required to produce 5-6 successive self-pollinations (which is the standard approach for breeders to consider germplasm as 'fixed'). It is estimated that up to 12 years may be required to produce inbred lines through this approach. Moreover, in the process of inbred line development, breeders generally take the opportunity to make selections in the successive segregating generations. In the case of cassava, unfortunately the breeder ends up selecting mostly for plants that flower, and not for plants that show adequate plant architecture, disease or pest resistance, and other traits related to good agronomic performance and productivity (particularly tolerance to inbreeding). It should be emphasized that in the case of cassava, the capacity to flower is not necessarily related to vigor. Many full-vigor and productive genotypes fail to flower. As a matter of fact, these non-flowering types offer the erect plant-architecture that farmers prefer. So, ultimately the pressure to produce lineages that have the capacity to flower would result in producing germplasm with undesirable plant architecture characteristics. Therefore, other approaches for the production of inbred germplasm must be considered.

6.1 Production of doubled-haploids through tissue culture

A very common approach to produce instant homozygosity is through anther (or less commonly ovule) culture. Immature pollen is harvested, subjected to some sort of stress and cultured *in vitro* in such a way that its biological pathway is changed. Eventually, cell division is induced in the microspore to produce a micro-callus, and from there embryos that can be induced to develop a plantlet. Because the explant is an immature microspore with half (N) the somatic number of chromosomes (2N) the tissue developed is haploid in nature. There is a frequent doubling of chromosomes that occurs spontaneously. The process, however, may have to be induced through the use of colchicine. This is the reason why this technology and the products it develops are known as doubled-haploids. The doubled-haploids technology is used extensively even for species that can produce 'fixed' lines in a matter of three years (maize and rice for example). Considering the reasons given above, the technology is more appealing in the case of cassava.

Work to develop the protocol for the production of doubled-haploids in cassava started in 2003 with a project supported by The Rockefeller Foundation. Early activities were directed toward the understanding of microspore development in cassava, so a uniform suspension of a large number of microspores at the right developmental stage could be obtained. Then the research faced an unexpected problem: the thick and autofluorescent exine wall of cassava microspore. The thickness of the wall prevented the observation inside the cells to see which treatment favored cell-division. The thick exine wall may have even prevented, by its pure physical strength, those cases where cell division had been initially induced to prosper and form a micro-callus. The autofluorescence prevented the use of special dyes to identify living from dead tissue. By the end of 2007 a methodology for degrading the exine wall was finally developed and this allowed for the routine development of multicellular structures.

The current work turns now around the final stage for the development of doubled-haploids: produce an embryo from the micro-callus, regenerate a viable plant and harden it so it can be transplanted to the field and be used to make crosses.

6.2 Production of doubled-haploids *in vivo*

There are examples reported in the literature where homozygous plants have been produced through sexual reproduction using wide crosses. The modes of origin vary, but in any case haploids arise following abnormal events during or soon after fertilization (frequently as a result of wide crosses between different species). The frequency of haploids is controlled by the genotype of the progenitors and there is an important influence of the environment, chemicals, timing of pollination and the effect of alien cytoplasm. Hermsen (1984) listed four different pathways for the production of haploid seed after sexual reproduction in different plant species: a) Pseudogamy; b) Preferential elimination of chromosomes; c) Semigamy; and d) Androgenesis.

With the exception of the exploitation of a gene in maize that allows for a predictable production of haploids or doubled-haploids *in vivo*, these systems are not as common as the *in vitro* approach. In the case of maize, 'inducer' lines have been developed to produce doubled-haploids *in vivo* (Röber *et al.,* 2005). . The use of an inducer line is a simple, fast, and inexpensive method of haploid production and is referred to as *in vivo* haploid induction.

6.3 Induction of flowering through exogenous applications of phyto-hormones

Flowering in cassava, as in every other crop, is controlled genetically. However, cassava is a perennial crop that does not need sexual reproduction for survival. The plant does not follow a pre-established phenological development (seed germination, vegetative growth, flowering, grain filling period, senescence and death) typical of many grain crops. Cassava shows marked genetic differences for flowering habit. Some genotypes will flower early and frequently leading to a branching type architecture. Other genotypes flower late and scarcely (or not at all), leading to non-branching, erect types. In several crops flowering can be stimulated by exogenous application of hormones (Botha *et al.,* 1998; Wilson *et al.,* 1990). The induction of flowering in pineapple has been known for a long time (Rodrigues, 1932) and the crop fits well for this kind of technology (Pinto da Cunha, 2005). Smoking was the first procedure used for artificial induction of pineapple flowering, after what may have been an accidental observation in the Azores Island. Later on it was discovered that the smoke agent that initiated the flowering was the gas ethylene. Ethylene is now applied commercially in pineapple production and has even allowed for some patents being granted in the way it is applied (U.S. patent 3819359).

The induction of flowering in cassava would offer very interesting alternatives, not only to facilitate (actually allow) the production of inbred lines through successive self-pollinations but also in the general operations of cassava breeding. When crosses between a set of progenitors are planned, it takes basically two years to obtain the botanical seed from these crosses. If a method to induce flowering in cassava breeding nurseries were available, then the time required to obtain the seed could be reduced considerably, perhaps to just six months.

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CHAPTER 7

USE OF BIOTECHNOLOGY TOOLS IN CASSAVA BREEDING

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INTRODUCTION

Cassava (*Manihot esculenta* Crantz), is grown primarily for its starchy tuberous roots and it is an important staple for more than 800 million people, mostly in sub-Saharan Africa, but also in other parts of Africa, Asia, the Pacific and South America. Cassava is an efficient producer of carbohydrate under sub-optimal conditions of uncertain rainfall, infertile soils and limited inputs encountered in the tropics.

Breeding goals of yield increases, root quality improvement, and disease resistance in cassava are considerably slowed down by biological characteristics of the crop, which includes a long growth cycle (8-24 months), vegetative propagation, perishability of the bulky roots, heterozygous genetic background and a poor knowledge of the organization of the crop diversity. These factors severely hamper the speed and ease of moving around useful genes in cassava. The consequences are that cassava production fails to keep up with demand, especially in regions where over 90% of the production is consumed as food, leading to an increase in acreage of cassava fields mostly into marginal lands.

Biotechnology can contribute to the solution of these problems and realize great benefits for cassava farmers. Cassava biotechnology through use of molecular markers, genome studies and plant genetic transformation have provided ways around breeding obstacles in long growth cycle and heterozygous crops. A number of these tools, including molecular genetic maps, markers linked to disease resistance genes, and marker aided studies of complex traits now exist. This paper highlights on achievements and progress made in cassava biotechnology in aid of the genetic improvement of the crop.

Tissue culture in cassava breeding

Cassava can be propagated either by stem cuttings or by sexual seed. Most breeding programs generate seeds through crossing as a means of creating new genetic variation. Crossing can be by controlled pollinations, done manually, to produce full-sib families or else in polycross nurseries where open pollination results in half-sib families. Tissue culture has been explored in cassava breeding through embryo rescue to improve the chances of germination especially for difficult crosses such as interspecific hybridization which often result in low fruit setting with minimal number of seeds. The vegetative multiplication rate of cassava is low. From one plant, 5-10 cuttings typically can be obtained, although it varies widely by genotype. This situation implies a lengthy process to arrive at the point where replicated evaluations across several locations can be conducted. It takes about 5-6 years from the time the botanical seed is germinated until the evaluation/selection cycle reaches the regional trial stage when several locations can be included. Tissue culture micro-propagation can rapidly facilitate the quick production of

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several copies of genotypes for the generation of sufficient planting materials for yield trials and multi-environment testing of genotypes. It could also facilitate the rapid generation of planting materials to farmers at the end of the breeding cycle. Recently, due to high risk associated with the use of stem cuttings for germplasm transfer, tissue culture has been used to facilitate germplasm transfer from the center of origin in Latin America to Africa to minimize the dangers and problems associated with disease and pest introduction. The National Root Crops Research Institute (NRCRI) in Nigeria has introduced under strict quarantine procedures through the Nigerian Agricultural Quarantine Service, several hundred genotypes of top elite lines for novel traits, such as high beta carotene and protein lines, using *in vitro* culture plantlets. The materials on receipt were micropropagated to generate more copies of each genotype before going through hardening, pre-nursery and finally to the field via transplanting. In addition, the use of *in vitro* germplasm has enabled several countries to share the same set of germplasm for multi-country testing of same genotypes or similar pedigrees in different ecologies for broad evaluation and genetic analysis. CIAT has been able to evaluate several genotypes shared with African countries such as Ghana, Nigeria, Tanzania, and Uganda supported by the Rockefeller Foundation and the Generation Challenge Programme (GCP).

Through tissue and cell culture technology, variation has been generated among *in vitro*-regenerated plants commonly known as somaclonal variation (Larkin and Scowcroft, 1981). This type of variation has yet to be fully exploited in cassava breeding but some success has been achieved in some crops with limited number of somaclonal variants being released as cultivars in a few crops such as banana, maize, and tomato. Point mutations, chromosomal rearrangements, recombination, DNA methylation, altered sequence copy number, and transposable elements are believed to be the basis of somaclonal variation (Kang *et al*., 2007). Several factors, such as genotype age of donor plants, type of explants, and age of culture, are reported to contribute toward this effect (Veilleaux and Johnson, 1998; Jain, 2001). Somaclonal variation mimics induced mutations and can be induced in both asexually propagated plants. It holds a lot of promise for plant breeding along with *in vitro* mutagenesis. Molecular understanding and availability of reliable markers for the detection of hypervariable DNA (hot spots which makes genotypes more prone to somatic variation) are key factors critical to the use of this approach as source of novel and unique genetic variation (Kang *et al.,* 2007).

Molecular markers

Molecular markers should not be considered as normal genes as they do not have any biological effect. Rather, they are constant landmarks in the genome. They are identifiable DNA sequences, found at specific locations of the genome, and transmitted by the standard laws of inheritance from one generation to the next. The markers differ in their technical requirements, with respect to the amount of money, time and labor needed, as well as the number of genetic markers that can be detected throughout the genome. These markers represent differences in the nucleotide sequences of either nuclear or organellar genomes and can be uncovered using diverse methods based upon PCR (Mullis, 1990), DNA-DNA hybridization (Botstein *et al.*, 1980; Fodor *et al*., 1993) or both. The most prominent markers include restriction fragment length polymorphisms (RFLPs; Botstein *et al*., 1980), random amplified polymorphic DNA (RAPDs; Williams *et al.*, 1990) markers, amplified fragment length polymorphisms (AFLPs; Vos *et al*., 1995), minisatellite (Jeffreys *et al.*, 1985), microsatellite, also called simple sequence repeats (SSRs; Litt and

Lutty, 1989a; 1989b), sequence characterized amplified region (SCARs) and single nucleotide polymorphisms (SNPs; Gupta *et al*., 2001). These markers have been used in cassava for genome mapping and gene discovery.

Genome mapping and gene discovery

Most agronomic traits are controlled by many genes, along with significant environmental influences. Molecular-based dissection of these complex traits, commonly known as QTL analysis, provides much more genetic information with which to make genetic advances. Valuable information from QTL mapping include: (1) the number, effect and chromosomal location of genes affecting traits; (2) the effect of multiple copies of individual genes (gene dosage); (3) non-allelic interaction between/among genes controlling a trait (epistasis); (4) the pleiotropic effects; and (5) stability of gene function in different environments (Paterson *et al*., 1991; Tanksley, 1993; Keasey, 2002). The Candidate genes approach (Faris *et al*., 1999) has been used to genetically dissect quantitative traits. In this strategy, one looks for certain biochemical pathways with possible involvement in the expression of that target trait. Based on the available sequence, information for a few genes should be retrieved and tested through mapping if the genes are linked to the QTL underlying target traits. Further linkage between genes and phenotype can be established by means of biochemical and genetic studies. Availability of high-density genetic and physical maps with linkage to ESTs and gene sequence may accelerate the identification of these candidate genes for success in MAS (Kang *et al.,* 2007). Markers have been used to generate several molecular genetic maps for cassava. Since the development of the first genetic map of cassava (Fregene *et al*., 1997) other new maps have since followed (Mba *et al*., 2001, Okogbenin *et al*., 2006; Kunkeaw *et al*., 2010; Sraphet *et al*., 2011).

The first genetic map (Fregene *et al*., 1997) was constructed based mainly on RFLPs, RAPDs, isozyme, candidate genes, AFLPs. In an attempt to make marker technology more widely applicable in breeding programs, highly polymorphic SSR markers were mainly used in the construction of subsequent genetic maps for cassava. Over 525 SSR markers have been used in the development of the new SSR-based maps which have yet to be published (Marin *et al*., unpublished; Hurtado *et al*., unpublished; Akinbo *et a*l., unpublished, Zarate *et al.*, unpublished; and Costatino, unpublished). An initiative towards completing the saturation of the cassava genetic map has also resulted in the generation of expressed sequence tags (ESTs) and SNPs. Several ESTs have been developed for cassava (Lopez *et al*., 2004; Lokko *et al*., 2007) with over 80,000 ESTs for cassava available in the Genbank. Over 1700 cassava SNPs are now available for molecular studies (Pablo *et al*., unpublished; Morag *et al.*, unpublished). SNPs are ideal markers as they allow the use of genotyping platforms that can assay many individuals for thousand of SNP markers in parallel. The strategy for utilizing markers is primarily driven by their availability and cost of genotyping platforms.

One of the primary objectives of genetic mapping and gene tagging efforts in cassava is to provide tools that can increase the cost effectiveness and efficiency of cassava breeding. Desirable characters that are difficult to evaluate using conventional methods are logical targets for molecular breeding of cassava. It includes pests and diseases, traits expressed only at the end of the crop's growing cycle and those for which phenotype is difficult to measure. Various markers have been used to tag several traits in cassava.

Molecular markers have been used to tag three different sources of CMD resistance (*M. glaziovii*, TME3 and TMS97/2205 (Fregene *et al*., 2000; Akano *et al*., 2002; Okogbenin unpublished data). Two SSR markers have been found associated with CGM (NS1009 and NS346). About six markers were found associated with CBB explaining 9-27% of the phenotypic variance of response to five *Xam* strains (Jorge *et al*., 2000). Early bulking is another trait evaluated in cassava and results from the analysis of this trait showed that it was mostly affected by harvest index and dry foliage. Three QTLs explaining 25-33% of phenotypic variance were found for dry foliage, while five other QTLs associated with harvest index with phenotypic variance in the range of 18-27% were identified (Okogbenin *et al*., 2002). This indicates that selection for HI in breeding scheme is an efficient indirect selection parameter for root yield.

In another study, bulked segregant analysis (BSA) was used to investigate the SSR markers associated with early bulking and high yield (EB-HY) in nine populations at NRCRI. Nine SSR markers (SSRY106, (ESTs)SSRY292, SSRY239, (ESTs)SSRY7, NS194, (ESTs)SSRY47, SSRY63, SSRY250, and NS323) were closely associated (r = 0.3-0.5; $p < 0.05$) with EB-HY in six of the nine F1 populations. The first seven markers with 10% or more coefficient of determination were linked to major quantitative trait loci associated with EB-HY in the cassava populations (Olasanmi, 2010). A total of 10 putative QTL were identified for protein (Akinbo, 2008). All the QTLs for protein content in the root showed a LOD score above 2.5. QTL found accounted for PVD ranges between 15% and 25% for protein content. All the QTL showed additive gene action with values ranging between 3.21 and 6.20. Except for CMD and CGM, markers identified for several traits are yet to be validated, and there is the need to test these markers and to further conduct fine mapping of the genomic regions for the markers with a view to developing better markers to enhance their application in marker assisted selection (MAS). Several other gene tagging projects have since been conducted or are on-going in cassava at CIAT and NRCRI for other traits such as PPD (Egesi *et al*., unpublished), whiteflies, and beta carotene (Marin *et al*., unpublished).

Cloning of genes

The heterozygous nature of cassava implies that attempts to introduce any trait, even when it is controlled by a single gene, may lead to the loss of a favored variety. A more efficient way to introduce traits controlled by a single gene, such as CMD2-resistance, is through genetic engineering. However, it is necessary to first clone the genes controlling the trait of interest. Genes, molecular tags and the knowledge accumulated can be used by plant breeders to address key cassava constraints that are not easily addressed through conventional tools (Fregene and Puonti-Kaelas, 2002). There are several approaches to cloning known only by its phenotype, or by its biochemical role in a biosynthetic pathway. The first is that of positional cloning (Martin *et al*., 1993; Tanksley *et al*., 1995) and cloning of genes via heterologous genes (Bothwell *et al*., 1990). The important criteria for positional cloning are a fine map based on a large mapping population of the appropriate genome region, a bacterial artificial chromosome (BAC) library and efficient transformation protocol for complementation analysis. A BAC library was constructed for cassava, for positional cloning of genes identified during genetic mapping of traits of agronomic interest (Fregene *et al*., 2000; 2001). Discovery of genetic markers linked to the CMD2 gene and the construction of a BAC library was initiated to facilitate positional cloning of the CMD resistance gene. Efforts are being made to add more markers

in the region of CMD2 resistance gene. Once fine maps have been obtained, a relationship between genetic distances and physical distances in the relevant regions will be estimated. Based on the estimated physical distance required to transverse the region, bearing the resistance gene, a BAC contig will then be constructed by BAC clone digestion and finger printing. Finally, the candidate BAC clones will then be introduced into cassava genotypes susceptible to CMD via genetic transformation.

Other genes of agronomic interest have also been cloned by the use of heterologous probes. One of the most important is the biosynthesis gene for the generation of cyanogenic glucosides. Two full length cDNA clones that encode cytochromes P-450, which catalyses the reactions in the biosynthetic pathway for cyanogenesis have been isolated using a heterologous probe (Anderson *et al*., 2000). Two cassava cytochromes, P-450 are 85% identical and share 54% sequence identity to CPY79A1 from sorghum and designated CY79D1 and CPY79D2. Both are actively transcribed in the cassava genome and production of acyanogenic cassava plants would therefore require down regulation of both genes.

Genes involved in the *in situ* break down of cyanogenic glucosides of cassava following tissue damage leading to the production of hydrocyanic acid have also been cloned. A linamarase cDNA clone (pCAS5) was isolated from a cotyledon cDNA library using a white clover beta-glucosidase heterologous probe (Hughes *et al*., 1992). Several genes controlling starch biosynthesis in cassava are also included in the list of cloned cassava genes (Muyinkwa *et al*., 1997). They include the AD glucose pyrophosphorase (AGPase) B and S gene that catalyses the synthesis of ADP glucose and the granule-bound synthethase (GBSSII) gene, the predominant starch synthethase gene that catalyses the conversion of AD-glucose to amylase. They were cloned using homologous genes from potato.

Several genes known to be involved in wound healing in plants have been characterized for their expression during post-harvest physiological deterioration (PPD). They include ACC oxidase that catalyses the last reaction of ethylene biosynthesis in plants, phenyl alanine ammonia-lyase (PAL), a key enzyme of the phenyl propanoid metabolism pathway, and catalase involved in the breakdown of hydrogen peroxide (Reily 2000).

Marker assisted selection (MAS)

(a) CMD resistance breeding

An ideal target for MAS is the breeding for disease resistance since one or few genes are often involved. MAS has rapidly facilitated the breeding for CMD resistance both in Latin America and in Africa where the disease is most prevalent. Breeding for resistance to CMD in Latin America, where the pathogen does not exist, requires the tools of MAS. The discovery of CMD resistance in TME3, a landrace from Nigeria, resulted in the development of molecular markers for this source of CMD resistance controlled by a single dominant gene designated as *CMD2* (Akano *et al*., 2002). Five markers are at present tightly associated to CMD2 with the closest being RME1 and NS158 at distances of four and seven cM, respectively, from the gene. MAS for CMD is being done using multiple flanking markers for genotyping activities (**Figure 1**).

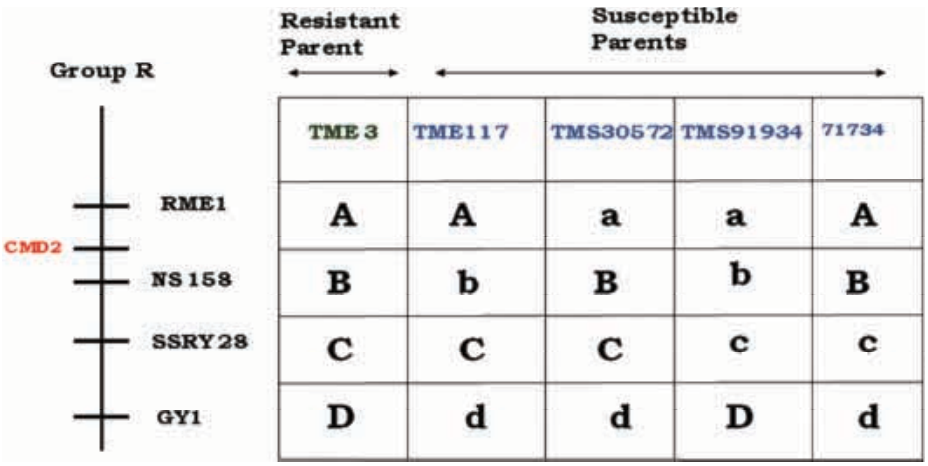

Figure 1. Selection based on multiple flanking markers for the CMD2 dominant gene.

The dominant nature and its effectiveness against a wide spectrum of the viral strains makes its deployment very appealing for protecting cassava against the actual and potential ravages of CMD in both Africa and Latin America (Blair *et al*., 2007). Field evaluations have indicated that RME1 and NS158 were excellent prediction tools for CMD resistance. A recent validation study indicate that MAS efficiency with these markers was around 68% (Okogbenin *et al.,* 2007). Through the success of MAS for CMD markers, several elite genotypes of Latin America were successfully introduced into Africa (Nigeria, Ghana, Tanzania, and Uganda) under the Generation Challenge Programme (GCP) research and capacity activities to improve and promote molecular breeding initiatives in African NARS. This has been a breakthrough considering that attempts to broaden the germplasm base of cassava in Africa using germplasm from Latin America (the crop's center of diversity) was largely impaired by the susceptibility of the germplasm to CMD, which is one of the two most serious disease constraint in Africa, the other being the cassava brown streak disease. Through MAS, a Latin American cassava variety, CR41-10 (UMUCASS 33) was released in 2010 and represents the first LA variety released in Africa. The need to improve CMD resistance and enhance its durability has further resulted in the screening for new sources of CMD resistance. Molecular marker analysis has identified a new source of CMD resistance. TMS 97/2205 has been found to show high CMD resistance in different ecologies with high to very high disease pressure in Nigeria (Egesi *et al.,* 2007). Results revealed that in an addition to the CMD2 which it possesses, an additional QTL with a PVE of 16% was involved in the genetic control of CMD resistance in this variety. The near immunity to CMD in this variety has been attributed to both loci. Efforts are ongoing to use SNP markers for fine mapping of both loci to improve MAS further for CMD. The detection of these CMD markers provides a prospect for pyramiding of CMD genes for high and durable CMD resistance in cassava. Through the integrated breeding platform established by the GCP, African NARs have started MAS programs for CMD resistance, thus opening further possibilities for other key important traits in cassava breeding with the availability of new genomic tools. In a conventional breeding program, the first 2-3 years

are used in screening for CMD resistance in particular before commencement of advanced yield trials. Through the use of CMD markers, the breeding scheme can be fast-tracked, meaning that varieties could be released in 5-6 years. Recently, over 1700 SNPs were developed in cassava (Pablo *et al*., unpublished; Morag *et al*., unpublished) and this is being used in several genetic mapping studies including those for CBSD, CMD and drought tolerance. There are on-going efforts to map additional CMD genes using 96/1089A as the source of new CMD resistance at NRCRI as part of the research activities of the GCP at NRCRI.

(b) Gene mining of wild relatives

Wild *Manihot* germplasm offers a wealth of useful genes for cassava (*Manihot esculenta*). Several accessions of *M. esculenta* sub spp. *flabellifolia*, *M. peruviana* and *M. tristis* have high levels of proteins (CIAT, 2004), Low amylase corn starch (3-5%) or waxy starch has been identified in *M. crassisepala* and *M. chloristicta*. Delayed post-harvest physiological deterioration has been identified in an interspecific hybrid between cassava and *M. walkerae* (Bertram, 1993). Moderate to high levels of resistance to CGM, white flies and the cassava mealybug have been found in interspecific hybrids of *M. esculenta* sub spp. *flabellifolia*. The use of wild species in breeding programs is restricted by linkage drag, which have accounted for the low use of the crop's wild relatives. Pre-breeding activities are therefore often required to allow for their easy and quick use in breeding programs. The long reproductive cycle and lengthy time requires to develop new cassava varieties (8-15 years) discourage the use of wild relatives in conventional breeding programs. The use of molecular markers to introgress a single target region of the genome can save two-four backcross generations (Frisch *et al*., 1999). In several crops, the tremendous genetic potential locked up in wild relatives has been released by the use of molecular genetic maps and the advanced backcross QTL mapping scheme (ABC-QTL) (Tanksley and McCouch, 1997). ABC-QTL has been explored in cassava, a process involving generating BC_1 and conducting QTL mapping followed by selection of genotypes carrying the genome of interest with minimum segment of the donor genome (**Figure 2**).
ABC-QTL have been used at CIAT to introgress genes for protein content, waxy starch and delayed PPD using polymorphic SSR markers, after which a QTL analysis was conducted using the phenotypic and molecular marker data. Genotypes with QTL of interest and minimum donor parent genome were then selected and used for generating advanced backcross populations (Blair *et al*., 2007). In the case of naturally occurring mutant granule-bound starch in wild relatives, a highly targeted approach was adopted. Sequencing of the glycosyl transferase region of the GBSSI gene from the wild relatives and two cassava accessions from cassava resulted in the identification of four SNPs, which differentiated the wild accessions from cassava. These were used to develop allele-specific molecular markers unique to these SNPs for selection of alleles in the breeding program (Blair *et a*l., 2007). Such allele-specific markers provide a huge opportunity selecting genotypes that bear the mutant gene for use in selfing in breeding programs to recover the waxy starch, which are recessive in nature. This approach represents an innovative molecular tool to accelerate the introgression of favorable alleles from wild relatives into cassava.

(c) *Genetic diversity*

Breeding programs depend on high levels of genetic diversity for achieving progress from selection. Broadening the genetic base of breeding populations require the identification of diverse genotypes for hybridization with elite cultivars (Xu *et al*., 2004;

Reif *et al*., 2005). Numerous studies investigating the assessment of genetic diversity within breeding material for cassava have been reported. DNA markers have been an indispensable tool for characterizing genetic resources and providing breeders with more detailed information to assist in selecting parents. It is not yet possible to predict the exact level of heterosis based on DNA marker data, although there have been reports of assigning parental lines to the proper heterotic groups (Lee *et al*., 1989, Reif *et al*., 2003).

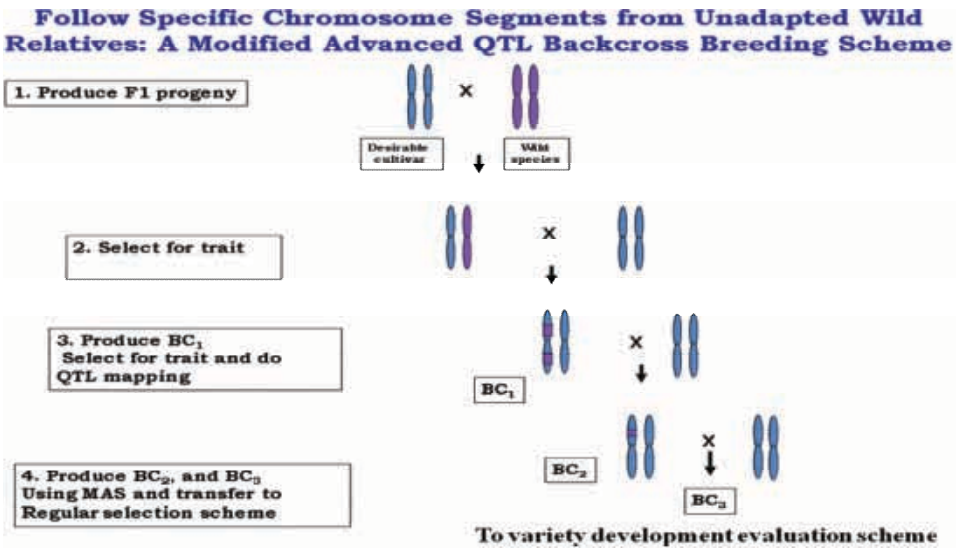

Figure 2. A modified advanced back cross QTL scheme used for the introgression of useful traits from wild relatives of cassava
***Source**: Blair et al., 2007.*

The genetic resources of cassava and its wild relatives represent a critical resource for the future of the crop. Germplasm collections and the study of genetic relationships between accessions have been made using molecular markers. Several markers (RFLP, SSR, AFLP, and DArT) have been used to date in genetic analysis of cassava germplasm. AFLP markers have been used for quantitative assessment of genetic relationships in representative samples of the crop's diversity and six wild taxa (Roa *et al*., 1997). In this study, *Manihot* species *Manihot esculenta* sub spp. *flabellifolia*, *M. trisitis* and *Manihot esculenta* were found to be more similar to cassava than its Mexican relative *M. aesculifolia*, indicating that cassava might have its origin in these close relatives (Roa *et al*., 1997). Evidence of introgression into cassava from *M. glaziovii* was also observed in an AFLP evaluation of genetic diversity in a large collection of cassava from the South American center of diversity (Second *et al*., 1997).

In other studies, markers have also been used to obtain a quantitative assessment of genetic similarity in cassava (Beeching *et al*., 1993; Second *et al*., 1997; Elias *et al.,* 2000) and to study the genetic structure of germplasm resistant to disease (Sanchez *et al.,* 1999, Fregene *et al*., 2000), including the genetic structure and the basis of genetic differentiation of cassava landraces in Africa (Mkumbira *et a*l., 2003; Fregene *et al*., unpublished data). Germplasm studies with markers have also revealed intravarietal polymorphism, indicating

that a variety could also be made up of more than one genotype (Elias *et al*., 2000). In a study by Mkumbira *et al*. (2002), genetic diversity was found to have been structured according to taste, bitter as against sweet varieties from Northern Malawi. Cassava appears to have highly differentiated gene pools and a large percentage of dominant/recessive gene loci, which are two key characteristics required for heterosis. Once the wealth of data has been analyzed, and crosses between clusters tested, hopefully, molecular markers can be used to predict heterosis. Markers have also been used to study the effect of disease on genetic diversity in cassava. Kizito *et al*. (2005) reported the loss of rare alleles in areas with high CMD incidence in Uganda. Genetic resources have been characterized at the regional (Fregene *et al*., 2003) and global (Hurtado *et al*., 2008) levels. Highly differentiated groups have been found among groups of materials from Guatemala and Africa and they may represent heterotic pools.

With new economic genotyping platforms that are designed for 96, 384 or 1536 SNPs per sample (such as Golden Gate from Illumina), genetic analysis of diversity is rapidly and efficiently being accomplished. A set of 200 accessions from Africa and Brazil have recently been analyzed for genetic assessment of diversity using SNPs at the ACGT, at the University of Pretoria (Myburg and Rabinowicz, unpublished). Under the Generation Challenge Programme, a total of 2568 accessions were used in a global genetic study using both SSR and DArT to build a composite set representing the range of diversity of cassava landraces and its wild relatives from a large gene bank (Fregene *et al*., unpublished). This study has resulted in the selection of a reference set for advanced challenges. This reference set will be useful for association mapping studies and functional diversity characterization. From genetic diversity studies conducted at CIAT, an SSR diversity kit of 36 SSR markers have been developed (Fregene *et al*., 2004) and are presently being used in breeding programs for genetic diversity analysis. Other applications include the use of markers for the identification of duplicates in germplasm (Chavarriaga-Aguirre *et al*., 1999) and the analysis of germplasm from the littoral and Amazonian regions of Brazil (Mueller *et al*., unpublished).

(d) *Estimation of heterozygosity during partial inbred line development*

Cassava is highly heterozygous and for much of the last four decades, inbreeding was not much practiced. Inbred lines are better parents for breeding and genetic studies as they do not have confounding effect of dominance and carry lower levels of genetic load (undesirable alleles). The speed of inbreeding depends upon the average heterozygosity of the original parental lines, the homozygosity level of the selected genotypes at the end of the self pollinating phase and the process of selection of progenies to be self-pollinated (Scotti *et al*., 2000). In the inbreeding process, phenotypically, there is a decrease in vigor, which is correlated with increased levels of homozygosity. The aim is to select vigorous plants (tolerant to inbreeding depression); in the process, plants may be selected that are less homozygous than the expected average for their generation. Selection in inbreeding is biased by the differences in homozygosity levels of segregating partially inbred genotypes (Blair *et al*., 2007). Molecular markers have been used to assess heterozygosity in cassava, enabling the selection of plants with true tolerance to inbreeding. About nine S_1 families at CIAT were assessed for heterozygosity using 100 mapped SSR markers distributed over 80% of the cassava genome, and evaluated for vigor, dry root yield and plant biomass (Blair *et al*, 2007). Recently in the GCP through the cassava breeding community of practice supported under the cassava challenge initiatives, there are on-going efforts to

assess for heterozygosity in over 20 S_1 and S_2 families in four countries (Nigeria, Ghana, Uganda and Tanzania) toward development of partially inbred lines. At each selfing generation, genotypes with the highest degree of homozygosity showing vigor will be selected and used for further selfing, and this will continue until high levels of homozygosity (i.e. least heterozygous) are attained with individuals showing tolerance to inbreeding depression leading to the identification of the best performing superior partially inbred parental lines. Molecular markers can be used to identify regions in the genome related to expression of heterosis and for measuring genetic distances in inbred lines to direct crosses with higher probabilities of high heterosis (Blair *et al.,* 2007) offering hope for maximization of hybrid vigor in cassava.

From MAS to Marker-assisted recurrent selection (MARS)

MAS is the selection of an individual with specific alleles for traits controlled by a limited number of loci (up to 6-8). MAS is desirable when phenotypic screening is particularly expensive and laborious. It is very useful for pyramiding multiple resistance genes. It is very useful when heritability is low. MAS has been successfully performed for many oligogenic traits (Garland *et al*., 2000; Murai *et al*., 2001; Jia *et al*., 2002; Komori *et al.*, 2003). But most agronomic traits are quantitative in nature and are the result of the joint action of several loci on a chromosome (QTL). To efficiently combine the best haplotypes for effective development of superior genotypes, maker-assisted recurrent selection (MARS) will best be suited to increase the frequency of favorable alleles based on a multi-parental strategy and using a "breeding by design" approach to recombine favorable alleles to build ideal haplotypes for target traits which are complex in nature. "Breeding by design" allows breeders to exploit known allelic variation to design superior genotypes by combining multiple favorable alleles (Peleman and van der Voort, 2003). MARS involves several cycles of marker-based selection and is effective in increasing the frequencies of favorable QTL or marker alleles. MARS is the identification and selection of several genomic regions (up to 20 or even more) for complex traits within a population. Under the Generation Challenge Programme activities, MARS is being implemented in the development of lines with improved production in the dry marginal environments in Africa, involving Nigeria and Ghana. In the MARS scheme being implemented by NRCRI, the first cycle will involve QTL identification and selection of individuals for favorable alleles, which are then used as parents for next crosses and then followed by one step of selection of best genotypes in the progenies based solely on markers. Under MARS, in subsequent selection cycles, the best genotypes are identified and are used as parents for the second selection cycle and this is subsequently repeated for the third selection cycle without phenotyping. Phenotyping will be done only after the third cycle of selection. This means that plants with the desired combinations of genes can be pre-selected before extensive and expensive field testing under MARS. It is expected that the best genotypes with good QTL combinations will result in good yield performance in drought ecologies. The development of exceptional drought tolerant varieties with durable pest/disease resistance requires identifying different QTLs in good combinations with the aid of markers, thereby increasing the frequency of developing ideal genotypes with best possible QTLs at each breeding cycle, and therefore the increasing probability of identifying and selecting a desirable genotype with ideal QTL combinations suited and adapted to the target agro-ecologies.

Genome wide selection

Genome wide selection (GWS) was found most useful for complex traits controlled by many QTLs and with a low h^2. It focuses more on the genetic improvement of quantitative traits rather than understanding their genetic basis. GWS can be implemented in the same way as MARS except that all individuals would have to be genotyped with a large number of markers. Genome wide selection (Meuwissen *et al*., 2001) focuses purely on prediction of performance based on as many loci as possible (unlimited number) and avoids QTL mapping altogether. GWS does not imply that QTL discovery should no longer be done, rather, the data used in GWS can be used to map QTLs (Bernardo, 2008). Therefore, GWS and QTL are not mutually exclusive. In GWS, the joint effects of all markers are fitted to random effects in a linear model. Trait values are predicted from a weighted index calculated for each marker. Simulation studies have indicated that across different numbers of QTL (20, 40 , and 100) and levels of h^2, responses to genome wide selection were 18 to 43% larger than the corresponding responses to MARS (Bernardo and Yu, 2007).

Genetic Engineering Improvement of Cassava Storage Roots

Beta carotene enhancement

Beta-carotene is a precursor of pro-vitamin A. Plants produce four pro-vitamin A carotenoids, distinguished by the possession of at least one retinyl group. Two of these molecules (α-carotene and β-carotene) accumulate in significant amounts whereas the others (γ-carotene and β-cryptoxanthin) are intermediates and tend to be converted rapidly into downstream products (Zhu *et al*., 2010). Pro-vitamin A carotenoids are synthesized *de novo* by plants. Increasing the availability of these compounds must involve metabolic engineering with the focus on β-carotene, because it is the most important and potent of the four available pro-vitamin A carotenoids. However, intervention can take place at any point along the pathway, and multiple strategies are available (Capell and Christou, 2004). General approaches include increasing flux through the entire carotenoid pathway by enhancing the production of GGPP, whereas more targeted approaches involve specifically boosting the production of β-carotene or reducing the amount of α-carotene, which can be regarded as a competitor because it shares a common precursor. As well as enhancing the synthesis of β-carotene, additional approaches include the inhibition of post-β-carotene steps to prevent conversion to zeaxanthin and other derivatives, and increasing the ability of plant cells to store β-carotene, thereby providing a metabolic sink and preventing feedback inhibition (Chao Bai *et al*., 2011).

Cassava is an important root crop in terms of carotenoid enhancement, because it is the preferred staple crop in some parts of Africa; but like cereals, it is generally a poor source of carotenoids. Cultivars with carotene-rich yellow roots are rare and most breeding populations have white roots (Ferreira *et al*., 2008; Nassar *et al*., 2009). Welsch *et al*. (2010) characterized the PSY2 locus in cassava and identified a polymorphism that increased carotenoid accumulation in cassava roots and also increased the rate of carotenoid synthesis when expressed in bacteria and yeast (Chao Bai *et al*., 2011). The strategy was the co-expression of codon-optimized *crtB* (with plastid transit peptide coding sequence) and the *Arabidopsis* 1-deoxyxylulose-5-phosphate synthase (DXS) genes, placed individually under control of patatin promoters. DXS was used in the second strategy to

increase total flux in the plastid isoprenoid pathway for enhanced production of the geranyl geranyl diphosphate (GGDP), the substrate of phytoene synthase. Because GGDP is also a precursor of the hydrophobic side chain of vitamin E tocochromanols, it was hypothesized that DXS upregulation would result in increased carotenoid production without impact on vitamin E concentrations in storage roots. Transgenic expression of phytoene synthase alone in cassava storage roots yielded increases in total carotenoid concentrations of 10- to 20-fold relative to amounts in roots from non-transformed controls. In these engineered roots, concentrations as high as 25 μg/g dry weight (DW) were detected. By comparison, carotenoid concentrations were 1 to 2.5 μg/g DW in storage roots from non-transformed plants.

Using the first strategy of co-expression of phytoene synthase and DXS transgenes, carotenoid concentrations in roots of similar age were 15- to 30-fold higher than those in storage roots from non-transformed plants, reaching concentrations of >50 μg/g DW. In the highest carotenoids producing roots, all-*trans*-β-carotene accounted for 85% to 90% of the total carotenoids content of storage roots, with minor amounts of lutein, 9-*cis*-β-carotene, and 13-*cis*-β-carotene. These studies from greenhouse-grown plants indicated that approaches involving the enhancement of flux into carotenoid biosynthesis are viable methods for provitamin A biofortification in cassava. Of the two strategies tested, co-expression of phytoene synthase and DXS was more effective at increasing carotenoids concentrations than expression of phytoene synthase alone (Sayre *et al*., 2011)

Protein content of cassava roots for nutritional enhancement

Cassava roots are an excellent source of starch and dietary energy but are very poor in protein (1-2% of dry weight). Increasing nutritional protein levels in cassava storage roots could beneficially impact diet and health in many regions, especially in Central and West Africa. As a first investigation to determine whether this may be feasible through transgenic technologies, researchers at ETH, Switzerland, genetically transformed plants of cv. 60444 with an artificial storage protein ASP1 gene, designed to be rich in essential amino acids (Kim *et al*., 1992). Analysis of regenerated tissues confirmed expression of the transgene at both the RNA and protein levels. Total protein content of *in vitro* leaves were found not to differ from the non-transgenic plants, but levels of the amino acids proline and serine were elevated and asparagine, alanine and methionine depressed compared to controls (Zhang *et al*., 2003c). Lack of vigor in these transgenic plants, most probably due to culture-induced somaclonal variation, prevented production of storage roots. However, these data demonstrate that it is possible to manipulate protein content in cassava through a transgenic approach. Moreover, new ASP1 transgenic cassava plants have been produced and phenotypically normal plants recovered. Strong expression of the ASP1 protein has been detected in the leaves from several of these lines growing in the greenhouse (ETH, unpublished results). It should be recognized that developing and deploying transgenic cassava with traits such as enhanced protein or other nutritional qualities presents significant technical and regulatory challenges.

Koster-Topfer *et al*. (1989) reported on cassava that was genetically modified using the patatin promoter to direct transgenic expression of zeolin to the tuberous roots. Zeolin is a fusion product between phaseolin, the major storage protein in common beans (*Phaseolus vulgaris*), and a truncated gamma-zein protein from maize (*Zea mays*), which directs the fused polypeptide to form stable protein bodies within the ER (Mainieri *et al*. (2004). According to Abhary *et al*. (2011), the production of transgenic cassava plants

expressing zeolin had storage roots with up to 12.5% of DW as protein, a more than fourfold increase compared to controls with no associated accumulation of protein in leaf tissues. Analysis of transgenic plants grown under greenhouse and field conditions have confirmed that this trait is stable when plants are propagated vegetatively; that it does not impact plant development, and that it is correlated with a significant reduction in the cyanogen content of both leaf and root tissues.

Reduced cyanogenic content in cassava

Cassava tissues accumulate cyanogenic compounds that can release cyanide into the body after ingestion. Biochemical studies have shown that the cyanogenic glycosides linamarin (95%) and lotaustralin (5%) accumulate in the vacuole while enzymes for their degradation are located in the cell wall. When tissues are crushed during processing or mastication these compounds are brought together and hydrolyzed to generate acetone cyanohydrin and glucose. Acetone cyanohydrin is then broken down spontaneously (at pH greater than 5 or temperatures above 35^{o}C) or by hydroxynitrile lyase (HNL) to produce acetone and hydrogen cyanide (White *et al*., 1998). Cyanogenic content is under strong genetic control and influenced by environmental conditions such as drought, varying between cultivars from 10 to 500 mg CN equivalents/kg dry weight in the storage roots (O'Brien *et al*., 1991). The lower end of this range exceeds FAO's recommended levels for food derived cyanide exposure. Proper processing prior to consumption is essential if the consumer is not to develop cyanide-induced disorders such as hyperthyroidism and ataxic neuropathy. Cassava has been utilized as a human food for millennia and the benefits derived from its consumption far outweigh any detrimental effects (Rosling, 1996). Therefore, there is a need to develop very low or even acyanogenic cassava. Decreased cyanogenic content in farmer-preferred cultivars would reduce the danger of exposure to cyanide by consumers and have potentially significant impact on commercial-scale cassava production. Overcoming such problems would improve cassava's economic competitiveness, develop as an industrial crop and provide increased income for cassava farmers. Recent research activities at Ohio State University, USA, and at KVL University, Denmark, have made significant progress towards the production of acyanogenic cassava. Two approaches have been taken. In the first, genes encoding a small (CYP79DI and CYP79D2) family of cytochrome P450s that catalyze the first dedicated step in linamarin and lotaustralin synthesis (Anderson *et al.*, 2000) were expressed in an antisense orientation in transgenic cassava of cv. MCol 2215. The second approach capitalized on earlier research from the same laboratory and also met with success. White *et al*. (1998) reported that levels of HNL in cassava storage roots were only 6% of that detected in leaves of the same plant, and commented that high residual acetone cyanohydrins in root tissues could be decreased by over-expressing HNL in the tissues, thereby accelerating the detoxification process and protecting consumers.

Manipulation of starch content

Several attributes of cassava's carbohydrate metabolism suggest that it has unrealized potential for enhanced starch production. For a C3 plant, cassava has an unusually high rate of photosynthetic carbon assimilation (43μmol $CO_2/m^2/s$) as well as a high temperature optimum (45°C) for photosynthesis (Hunt *et al*., 1977; Edwards *et al.*, 1990; Angelov *et al*., 1993). In addition, cassava has been reported to have one of the highest rates of CO_2 assimilation into sucrose of any plant measured (Hunt *et al*., 1977;

Angelov *et al*., 1993). For these reasons, cassava is an excellent candidate for enhancing carbohydrate allocation to sink tissues through transgenic approaches. Increase in sink strength has been achieved by the expression of a modified bacterial ADP-glucose pyrophosphorylase (AGPase) gene in cassava tuberous roots. AGPase plays a critical role in the regulation of starch synthesis in plants, not only because it catalyses the first dedicated step in starch synthesis, but also because it is the rate-limiting step in starch synthesis. Antisense-mediated inhibition of AGPase expression has been shown to lead to a severe decrease in starch production in potato tubers (Muller-Rober *et al*., 1992) as well as in cassava tuberous roots (Munyikwa *et al*., 1998). AGPase have been characterized from several different species and have been shown to have different structures, catalytic rates and allosteric regulation. The plant's AGPase holoenzyme is a heterotetramer and is formed from two distinct polypeptides which comprise the large and small subunits. These requirements make the genetic manipulation of the plant AGPase more challenging, as it potentially requires modification of the expression or activity of one or more AGPase genes in transgenic plants. In addition, the plant AGPase is activated by 3-phosphoglycerate (3-PGA), inhibited by inorganic phosphate (Pi) and regulated by the redox state of the cell (Ballicora *et al*., 2000; Tiessen *et al*., 2002; Geigenberger, 2003).The bacterial AGPase is allosterically regulated by effectors different from those of the plant AGPase. The bacterial AGPase is activated by fructose-1,6-bisphosphate (FBP) and is inhibited by adenosine monophosphate (AMP) (Preiss, 1988).The *Escherichia coli* (bacterial) AGPase is also a single gene(glgC) product and, importantly, its specific activity is several hundred-fold greater than that of the plant enzyme. Several residues have been identified as important allosteric regulatory sites (Kumar *et al*., 1989; Frueauf *et al*., 2001. A glycine-336 mutant (G336D) has been shown to have high activity with or without the activator FBP, higher substrate (ATP and glucose-1-phosphate) affinity and reduced affinity for the inhibitor AMP (Meyer *et al*., 1998).

According to Ihemere (2003) the starch biosynthesis capacity of cassava by enhancing the enzyme activity of ADP-glucose pyrophosphorylase (AGPase) was increased by the integration of the TP/glgC gene driven by patatin promoter transformed into cassava target tissue. The transgenic lines expressing the TP/glgC gene were shown to express the TP/glgC gene only in root and not in leaves. The AGPase enzyme activity analyses indicated that the transformed plants had between a 65% and 95% increase in AGPase activity relative to wild type. The relative differences in AGPase activity between transformants is as the result of position effects or the site of insertion of the transgene into the genome (Zhang *et al*., 2000a; Sarria *et al*., 2000) or variation in copy numbers of the TP/glgC copies inserted into the cassava genome. The transformed plants (3D-1 and 3D-3) having two copies of the glgC gene had higher AGPase enzyme activity and higher root yields compared to the transformed plant (3D-2) with only one copy of bacterial glgC gene. The relative increase in AGPase activity compared to wild type was less than that observed for expression of the HNL gene in transgenic cassava roots (also driven by the patatin promoter) (Siritunga *et al*., 2003). However, the base-line level of AGPase activity in wild type cassava roots is substantially higher than that of HNL. The absolute AGPase activity of wild-type and transgenic cassava was similar to that reported for other species (Ihemere *et al*., 2006).

Zinc biofortification of cassava storage roots

The micronutrient zinc (Zn) is essential for all organisms (Broadley *et al*., 2007; Andreini *et al*., 2006). The element is required as a cofactor in over 300 enzymes (Coleman, 1998) and plays critical structural roles in many proteins, including countless

transcription factors (Hershfinkel, 2006; Kramer and Clemens, 2006), increasing the Zn concentration of food crop plants, resulting in better crop production and improved human health. Zn deficiency occurred in both crops and humans (White and Zasoski, 1999; Hotz and Brown, 2004; Welch and Graham, 2004). According to a WHO report on the risk factors responsible for development of illnesses and diseases, Zn deficiency ranks 11th among the 20 most important factors in the world, and 5th among the 10 most important factors in developing countries. In a comprehensive study, Hotz and Brown (2004) reported that Zn deficiency affects, on average, one-third of the world's population, ranging from 4 to 73% in different countries. Zinc deficiency is responsible for many severe health complications, including impairments of physical growth, immune system and learning ability, combined with increased risk of infections, DNA damage and cancer development (Hotz and Brown, 2004; Gibson, 2006; Prasad, 2007). Increasing the amount of bioavailable micronutrients in plant foods for human consumption is a challenge, which is particularly important for developing countries. This could be achieved by increasing the level of micronutrients in the edible part of staple crops.

Cassava (*Manihot esculenta*), being the major staple food crop for more than 300 million people in Africa lacks important micronutrients such as Vitamin A, iron and zinc**.** Genotype-environment interaction studies suggest that variation in Zn concentration in cassava roots is due mostly to the soil available Zn level and soil pH (CIAT, 2006). Although genotypic variation for Zn has been reported (Chavez *et al*., 2005), the zinc concentration in the tuberous roots can be increased more than fourfold by overexpressing vacuolar- and plasma membrane-localized zinc transporters (Grotz *et al*., 1998; Kobae *et al.*, 2004). Overexpression of the vacuole membrane localized *ZAT* gene had previously been shown to elevate zinc levels in plants and was used to increase the tuberous root zinc concentration fourfold (Gaitan-Solis *et al*., unpublished data) through the targeted over-expression of the *Arabidopsis* ZAT transporter using the patatin promoter (Siritunga Sayre, 2003). An *Arabidopsis* ZIP plasma membrane zinc transporter (Grotz *et al*., 1998) was also over-expressed in cassava to increase tuberous root zinc content. Cassava tuberous root zinc concentrations were enhanced two- to ten-fold. However, leaf zinc concentrations were reduced and the leaves of the transgenic plants appeared zinc deficient (Gaitan-Solis *et al*., unpublished data). The higher zinc concentrations in roots and lower concentrations in leaves in cassava due to over-expression of a ZIP transporter were similar to what was observed. Over-expression of zinc transporters has been successful in altering the zinc concentration of multiple tissues in several plant species. However, the reduced zinc concentration in cassava and rice leaves and decreased grain yield in rice caused by transporter over-expression highlight the need to further refine this approach (Sayre *et al.*, 2011).

In other studies, S. Kahya and N. Narayanan (unpublished data) hypothesise that ZIP driven by patatin promoter has created a major sink in the root tissue, which leads to uneven distribution of zinc in the shoots. Therefore, the strategy tested for over-expressing ZIP gene driven by *Arabidopsis* A14-root epidermal promoter indicates that enhanced zinc homeostatic distribution throughout the cassava plant has increased the root to shoot translocation of zinc via the root and epidermis leaves and not concentrated in the root cortex

Post harvest physiological deterioration

Cassava storage roots suffer from a rapid deterioration upon harvest, which can reduce considerably the palatability and marketability of cassava roots within 24-72 hours after harvest (Ndunguru *et al*., 1998; Janssen, 1985; Wenham, 1995), a phenomenon referred to as post-harvest physiological deterioration (PPD). This is a major constraint to the development and exploitation of cassava as a crop, food, and commodity. It is characterized by a blue-black discoloration of the xylem vessels known as "vascular streaking" The onset of PPD is associated with an increase in respiration (Uritani, 1998; Hirose, 1984), changes in lipid composition (Lalaguna and Agudo, 1989), synthesis of ethylene (Hirose, 1984). accumulation of secondary metabolites from the phenylpropanoid pathway, and increases in many enzyme activities, including PAL and chalcone synthase, glucanase, chitinase, proteinase inhibitors, HRGPs, invertase, catalase, dehydrogenase, peroxidase, and polyphenol oxidase (Rickard, 1981; Tanaka *et al*., 1983). Wounding of cassava roots enhances respiration rates within the first day, which is followed by primary physiological deterioration (Uritani, 1998; Uritani and Reyes, 1984). The phytohormone ethylene is produced in cassava roots within 6 hours of wounding (Plumbley *et al*., 1981; Hirose *et al.*, 1984), with higher ethylene production in susceptible roots. Experiments utilizing cycloheximide to inhibit protein synthesis (Uritani and Reyes, 1984) and other studies (Beeching *et al*., 1998) have shown that PPD is an active process involving gene expression and protein synthesis. There are 72 differentially regulated ESTs, of which 63 were upregulated and 9 were down-regulated. Many of the upregulated PPD-specific ESTs were predicted to play roles in cell wall repair, reactive oxygen species (ROS) generation and turnover, programmed cell death, ion/water/metabolite transport, signal transduction, stress response and metabolism, and protein synthesis (Reilly *et al*., 2007). ROS have been shown to increase earlier during PPD (Reilly *et al*., 2003). And evidence for the involvement of ROS and associated turnover enzymes during PPD is accumulating (Chavez *et al*., 2000; Reilly *et al*., 2007). Several lines of evidence suggest that there is a controlled production of ROS in plant defense, especially in response to wounding and pathogen attack. Reilly *et al.* (2007) reported a rapid oxidative burst within 15 min of harvest, signaling the start of PPD, predominantly due to a rapid production of superoxide and hydrogen peroxide (Reilly *et al*., 2003). Several roles have been attributed to the accumulated ROS species, among which cell wall repair and remodeling, induction of defense-related genes, signal transduction, and triggering host cell death are significant. Using sequentially sectioned cassava roots it was found that superoxide dismutase (SOD), catalase, and peroxidase were predominantly expressed in regions closer to the wound site (Iyer *et al.*, 2010).

Based on the great evidence that an oxidative burst was associated with the onset of PPD, two different strategies were developed to reduce PPD: prevention of ROS production and scavenging ROS. According to Maxwell *et al*. (1999) PPD could be controlled by reducing cyanide-dependent ROS production and accumulation. The over-expression of *Arabidopsis* AOX in transgenic cassava roots resulted in substantially reduced ROS accumulation and delayed the onset of PPD by as much as three weeks, enough time for the shipping or processing operations necessary after harvesting the crop. The second strategy to reduce PPD was to quench ROS production by over-expression of ROS-metabolizing enzymes (e.g. catalase, SOD, ascorbate peroxidase) or by the over-accumulation of anti-oxidants, such as β-carotene. It was also previously observed that cassava varieties with elevated β-carotene content had an extended shelf life (Gloria and Uritani, 1984). Indeed, the shelf life of transgenic plants with elevated β-carotene (40 ppm)

content was extended to four weeks. Overall, these results suggest that ROS production from cyanide-poisoned mitochondria initiate PPD and that reduction in ROS accumulation will extend the shelf life of harvested cassava roots (Sayre *et al.,* 2011).

Increasing Iron content in cassava storage root

Iron is one of the most abundant elements in the earth's crust, but it is considered the third most limiting nutrient for plant growth owing to its low solubility (Jeong and Guerinot, 2009). Iron concentrations (10 ppm) in cassava storage roots are insufficient to meet the minimum daily requirement of humans. The strategy used to increased iron in cassava roots, including increasing iron uptake using an iron-specific assimilatory protein (FEA1) from *Chlamydomonas reinhardtii*; overexpression of the iron storage protein, ferritin; and a combination of both strategies (Narayanan *et al*., 2011; Rubinelli *et al*., 2002).The three strategies effectively gave similar results. The results obtained using the FEA1 iron assimilatory protein, because it is an iron-specific assimilatory protein and is operational in high-pH soils, limits iron uptake (Narayanan *et al.,* 2011). Expression of the *FEA1* gene in wild-type *Arabidopsis* was shown to increase the root iron concentration eight-fold (Narayanan *et al*., 2011). A codon-optimized *FEA1* gene was expressed in cassava roots under control of the patatin promoter. The iron concentration of young (2 month old), wild-type fibrous roots was approximately 800 ppm, but by the time the roots had fully expanded the iron content had been reduced nearly 100-fold (Ihemere *et al*., 2011). This progressive decrease in iron content with age was presumably a result of tuberization (starch accumulation) and a reduction in iron uptake associated with the loss of root hairs in storage roots. The root iron content of the best performing transgenic lines was 42 ppm at 6-month of age. Importantly, no secondary phenotypic effects were observed in greenhouse- or field-grown plants. Preliminary analysis of the expression levels of genes involved in iron homeostasis indicated that ferritin expression increased six-fold in roots of transgenic plants expressing the *FEA1* gene. As cassava contains virtually no detectable phytic acid, it is expected that ferritin-associated iron will be very bio-available (N. Taylor, unpublished data).

Cassava mosaic disease.

Cassava is vegetatively propagated and is vulnerable to viral infections. Cassava brown streak disease (CBSD) and cassava mosaic virus (CMD) are the two common cassava diseases in Africa. CMD is caused by a cassava mosaic geminivirus, and is transmitted by whiteflies [*Bemisia tabaci* (Alicai *et al*., 2007, Legg and Thresh, 2000; Patil and Fauquet. 2009)].The two components of cassava geminiviruses are DNA-A and DNA-B, which encode a total of eight viral proteins. DNA-A encodes six proteins involved in replication, transcription and encapsidation, whereas DNA-B encodes two proteins required for virus movement (Gutierrez, 2000; Hanley-Bowdoin *et al*., 2004). In the last two decades, various approaches have been tested in plants to engineer geminivirus resistance (Rickar, 1982.). Strategies tested were mostly based on involvement in key viral protein functions such as the Rep associated protein, which is required for replication. It has been discovered that gene silencing play a part in plant defenses against viruses (Voinnet, 2001), which opens new routes to engineer virus resistance via RNA interference (RNAi) pathways. Pooggin *et al*. (2003) have shown in previous studies that transient expression of hairpin double stranded (ds) RNAs homologous to the noncoding intergenic region of *Vigna mungo* yellow mosaic virus could enhance plant recovery from viral infection. Stable transgenic cassava lines over-expressing hairpin dsRNAs homologous to the non-

coding intergenic region of African cassava mosaic virus (ACMV) were produced. The cassava lines remained susceptible to ACMV infection, but they had an enhanced recovery phenotype compared with wild-type plants (Van der Schuren *et al*., 2007). ACMV replication appeared to be strongly impeded in leaf disks of transgenic cassava lines. Due to its key role in viral replication, the viral replication-associated protein (Rep) appeared as an obvious target for silencing (Zhang *et al*., 2005). Constitutive expression of artificial hairpin dsRNAs homologous to the Rep coding sequence in transgenic cassava demonstrated that an ACMV-susceptible cultivar could become immune to ACMV infection (Van der Schuren *et al*., 2009). Virus resistance levels correlated with the load of hairpin-derived small RNAs.

Doubled Haploid Technology

Doubled haploids (DH) have yet to be developed in cassava, but research efforts are underway to enhance the possibility of utilizing this technology in cassava. Doubled haploids offer manifold advantages to the plant breeder because of rapid fixation of homozygosity in just one generation, as against five to six generations in conventional breeding in some crops, increased selection efficiency, especially for recessive traits (Kang *et al*., 2007). Because DH lines are homozygous, with no segregation in subsequent generations, they are very useful in genetic studies involving identification and mapping of major genes/QTL. This reduces the environmental component of the total variation, thereby allowing the precise measurement of quantitative traits by repeated trials (Lu *et al*., 1996). Brennan (1989) showed that the use of DH technology can reduce the time to release new varieties and increase the economic value of the outputs of a breeding program by 20-30%. In addition, shortening the breeding cycle means that the rate of genetic gain on farms over a number of cycles is increased. The value of time-reducing technologies that accelerate the breeding process varies by program and the level of production based on varieties (Brennan and Martin, 2007).

The Future

Biotechnology tools have rapidly contributed to the pace of cassava genetic improvement by enhancing the genetic knowledge of the crop and the deployment of useful information in the breeding process, in addition to facilitating and expediting the breeding process for the fast-track release of improved varieties to farmers.

Advances in genomics have improved the efficient use of molecular markers in breeding programs through their use in marker-assisted selection strategies. Their use in breeding has been able to address challenges associated with: traits that are difficult to manage in conventional phenotypic selection; traits whose selection depend on specific environments; maintenance of recessive alleles in backcross schemes; and pyramiding of monogenic traits (Xu and Crouch, 2008). The many steps in developing appropriate MAB approaches can be very daunting, as they are often demanding in time, efforts and expenses. However, once a good knowledge base is created to estimate appropriate parameters, which efficiently determine the trait, a good experimental set up could result in the improvement of marker-assisted breeding tools, which can, to a major extent, minimize future applications of phenotypical assays (Peleman and Van der Voort, 2004).

Inspite of the significant achievements that have been facilitated by the use of markers in genetic analysis and crop improvement, the rapid integration of markers in breeding still need to be improved upon to make the gains of such applications result in

massive investment returns in products developed through such processes. Molecular markers are highly reliable selection tools, not influenced by the environment, and relatively easy to score in the laboratory. Reducing the cost of molecular assays relative to phenotyping costs is one of the key considerations in the application of this technology in breeding. Cost benefit analysis is critical for the successful implementation of marker assisted breeding. Over time, there has been a considerable reduction in cost with high throughput technology rapidly making marker assays much cheaper. When markers are applied in the most efficient combination of multi-pooling and multiplexing, it could significantly lead to reduced costs. Higher throughput systems will have lower costs per assay, as the capital and overhead costs per sample will be lower (Brennan and Martin, 2007).

The Generation Challenge Programme recently supported the establishment of an integrated breeding platform to support marker assisted breeding programs in research centers at an efficient speed and to save cost associated with developing labs' state-of-the-art equipment, which often times is prohibitive in public funded research centers. The Integrated Breeding Platform seeks to reproduce and replicate the success attained in the private sector in the public sector and to improve capacity of MAB in developing countries.

There has been marked improvements in the use of tissue culture to rapidly overcome the low multiplication rate of cassava, and thereby facilitating the rapid generation of planting materials for multi-locational breeding trials required in the breeding program – a process that could shorten the breeding cycle significantly. While this is a huge step in the breeding program, the need to develop the doubled haploid technology is a key challenge that could help to change the face of cassava breeding if successfully achieved.

The use of transgenic technologies to incorporate desired traits into farmer preferred cultivars and landraces, as well as elite breeding lines, is of paramount importance, especially for rare novel traits of high significance to end-users. Further improvements in the ability to transfer new genetic materials into the cassava genome using this approach is essential if the crop is to fully benefit from major advances occurring in the genomic and post-genomic era. A key challenge for the cassava biotechnology group is to develop efficient transformation protocols that can be used with farmer-preferred cassava cultivars.

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CHAPTER 8

USE OF TISSUE CULTURE FOR GERMPLASM CONSERVATION AND TRANSFER

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INTRODUCTION

The collection, characterization, and utilization of genetic resources are very important for global agricultural needs. This biodiversity needs to be conserved to mitigate possible natural disasters against extinction and to ensure their future availability. The fundamental objective of genetic resources conservation is the maintenance of broad based genetic diversity within each of the species (i.e., intra-specific genetic diversity) with a known or potential value in order to ensure availability for exploitation by present and future generations. Plant genetic resources can be classified into the following groups, such as: (a) advanced varieties in current commercial use and bred varieties no longer in commercial use; (b) genetic stocks, i.e., lines that carry particular mutations, cytogenetic rearrangements, or linkage markers; (c) bulk populations or composite crosses developed from crosses from a wide variety of cultivars; (d) landraces associated with traditional, pre-scientific agriculture; and (e) wild progenitors or relatives of potential use in crop breeding (or as new crops)

Germplasm is the potential hereditary stocks within a species, taken collectively, that is used by plant breeders to develop new cultivars. Plant breeders require genetic variation (gene pools) for crop improvement. The higher the variation, the better the chances of breeding for key strategic traits of economic importance, such as biotic and abiotic stresses which are critical for good adaptation to wider ecological amplitudes. However, in the wake of the spread of high yielding varieties, this genetic variability may be undermined leading to large-scale depletion of variability. This situation thus demands priority action to conserve such germplasm (Frankel, 1975). The need to avert genetic erosion means that germplasm must be conserved in such a manner that there are minimal losses or changes in genetic variability of the population. This entails determining the most appropriate storage methods suitable for germplasm conservation, whether seeds, pollen, roots, tubers, bulbs, other vegetative material or cell, meristem and other tissue culture systems.

Gene banks (seed banks) are generally advocated, since the storage technology is relatively simple and well known at least for most annual, orthodox seed species which are desiccation tolerant. The demands of seeking an effective strategy for collection and conservation of samples of crops that are normally propagated vegetatively, or that produce seeds which cannot be stored using normal procedures of storage require special alternative methods. This has necessitated the need for tissue culture or *in-vitro* techniques for germplasm conservation (Withers and Alderson, 1986). Tissue culture is the growth of tissues and/or cells separate from the organism. This is typically facilitated via use of a liquid, semi-solid, or solid growth media, such as broth or agar. It generally refers to the growth of eukaryotic cells *in vitro,* and this has been widely applied to the culturing of tissue pieces, i.e. explant cultures or whole organs, i.e. organ culture. Tissue culture *or in vitro* techniques have great potential for collecting, exchange and conservation of: genetic

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resources of recalcitrant-seed and vegetatively propagated species as well as of endangered species; elite genotypes (which are multiplied on a large scale in production laboratories) and cultures with special attributes (e.g. metabolite-producing cell lines and genetically engineered material).

In vitro cultures are also routinely used for exchange of plant genetic resources of a number of species, due to their advantages in terms of phytosanitary status and reduced cost. Slow growth techniques have been developed for medium-term conservation of numerous species but their routine use is still restricted to a limited number of crop species. Routine use of cryopreservation is mostly restricted to conservation of cell lines in research laboratories. However, simple and efficient freezing protocols have been developed recently for apices and embryos, and can be considered operational for an increasing number of species.

I*n-vitro* storage of germplasm has been used to address key issues related to cycling of the material through multiplication schemes, distribution of germplasm and also its characterization and evaluation. Hence, the development of the full potential of *in-vitro* culture storage and associated biochemical techniques has immensely revolutionized the handling of germplasm.

A range of *in-vitro* techniques have been developed in the last few decades. The organized culture systems have a high degree of genetic stability and are more likely to be of importance for germplasm storage, especially the 'shoot tips' or meristem cultures. This paper highlights on the increasing importance of tissue culture and biotechnology in germplasm conservation and transfer for access by breeders for global crop improvement to meet the needs of mankind and its ability to be able to respond to the ever increasing evolutionary changes and challenges that threatens to undermine current efforts to meet world food demands.

Genetic Erosion

Germplasm is the genetic source material used by plant breeders to develop new cultivars. Germplasm may include seeds or other plant parts as a leaf, stem, pollen, or cultured cells that could be grown into mature plants. Genetic erosion in agricultural biodiversity is the loss of genetic diversity, including the loss of individual genes, and the loss of particular combinants of genes (or gene complexes) such as those manifested in locally adapted organisms adapted to the natural environment in which they originated. The term genetic erosion is sometimes used in a narrow sense, such as for the loss of alleles or genes, as well as more broadly, referring to the loss of varieties or even species. Maxted and Guarino (2006) define genetic erosion as the permanent reduction in richness (or evenness) of common alleles, or the loss of combinations of alleles over time in a defined area. Many researchers believe that the main problem related to agro-ecosystem management is the general tendency towards genetic and ecological uniformity imposed by the development of modern agriculture.

The major driving forces behind genetic erosion in crops are: variety replacement, land clearing, overexploitation of species, population pressure, environmental degradation, overgrazing, policy and changing agricultural systems. There is a high rate of biodiversity loss, damage or injury due to high commercial and social demand, urbanization, land use and climatic events, agricultural intensification. Some species are further threatened by overexploitation/over-harvesting of natural stands as well as land use change. Occurrence of destructive pests and disease as well as natural calamities and the use of few genotypes contribute to the rapid loss of germplasm. Changes in relative importance of major crops in

countries influence prioritization of conservation of those crops. These changes are due to land conversion, increase in volume of export due to increased global demand, pests and diseases, expanded use and new markets, importation and competition with other crops (Illa and Catibog, 2008). Unstable peace and order situations arising from conflicts are also serious threats to biodiversity and hence need for conservation. Ong *et al.* (2002) cautioned that every parcel of land that is converted, cultivated or developed poses a risk to existing biological resources.

Landraces are also threatened by the replacement of landraces with commercial hybrids. Breeders use landraces and modern varieties mainly for their breeding activities. Landraces (farmers varieties) are an earlier cultivated form of a crop species, evolved from a wild population and adapted to certain conditions. They are highly heterogeneous populations of crop plants grown by traditional farmers resulting from thousands of years of selection (natural/human). On the other hand, modern varieties, obtained by plant breeding, are genetically homogeneous populations highly adapted to modern uniform agricultural management techniques. This genetic uniformity is the result of intensive selection for the genotype that best meets modern production and market needs. However, the selection and purification of landraces lead to more uniformity and less genetic variability within the improved cultivars during the period of plant breeding, leading to genetic erosion.

Destruction of natural habitats threatens the continued existence of natural populations of wild species. The choice to grow only the preferred varieties under monocropping can contribute to the loss of genetic variability of the wild species. If only one commercial cultivar is grown wide-spread as in the monocropping or monoculture, there will be no source of new resistant genes in this plant species to develop to fight the new diseases. The loss of genetic diversity contributes to genetic vulnerability – a condition when a crop is uniformly susceptible to a pest, pathogen or environmental hazard as a result of its genetic changes. Significant loss of genetic diversity during crop domestication has also been reported (Tanksley and McCouch, 1997). A major cause of loss of genetic diversity – referred to as genetic erosion – has been the spread of modern, commercial agriculture. The introduction of new, highly uniform varieties has resulted in the loss of traditional farmers' varieties. Therefore, the objectives of germplasm conservation are: to prevent the loss of ‘domesticated’ genes, to introduce ‘wild’ genes into culture. Increasing human expansion and deforestation, which are problems related to overpopulation, are serious threats to plant genetic resources and may cause important loss to the gene pool. A rich ecological environment is indeed very complex, and is impossible for humans to recreate. Genetic erosion can wipe out millions of years of evolution and a loss in biodiversity is not something we can bring back.

Germplasm Conservation

Germplasm conservation can be at ecosystem level, genotype (*ex situ* level), or gene (molecular) level. The strategies used are determined by the scope (**Table 1**) and biological considerations (**Table 2**). The biological considerations can also strongly influence the conservation strategy used as determined by several factors such as the type of plant species, propagation materials etc. There are broadly speaking two basic approaches to genetic resources conservation, namely, *in-situ* and *ex-situ* conservation. The choice of *in-situ* and *ex-situ* conservation is sometimes seen in terms of exclusive alternate strategies, but the two alternatives may be more constructively viewed as mutually

complementary activities, and each can play an important part in safeguarding particular plant populations. It would be an ideal situation where both may be used to best advantage to ensure both long-term species survival and an adequate supply of germplasm for improvement of related crops.

Table 1. Methods of conservation of plant genetic resources.

Methods	**Predominantly conserved PGR categories by corresponding method**
Biosphere reserve	Ecosystem/biodiversity by and large
Nature reserve	Specific habitat/wild and/or weedy species gene pool
Gene sanctuary	Ecosystem (specific)/ wild species gene pool
On farm conservation (mass reservoirs, bulk hybrid populations)	Agro-ecosystems/land races
Botanical garden/arboretum	Wild species, obsolete cultivars, tree crop germplasm
Field gene bank	Wild species, vegetatively propagated crops, tree crop germplasm
Plant organ storage	Vegetatively propagated crops, mainly in the form of roots, tubers and bulbs
Seed storage	All plant species which produce fertile and othodox seeds
Pollen storage	In principle all species which produce long living pollen
In vitro storage	Wild and cultivated species which produce recalcitrant or no seeds, vegetatively propagated crops, disease free germplasm as well as orthodox seeds
Cryopreservation	Germplasm mentioned above which permits cryopreservation
DNA and gene libraries	Special genetic stocks: in principle applicable for all germplasm

Source: *S.D. Shikhamany.*

In-situ Conservation

In-situ means the setting aside of natural reserves, where the species are allowed to remain in their ecosystems within a natural or properly managed ecological continuum. The natural biosphere reserve is a useful solution for species that are endangered and nearly on the point of extinction (Prescott-Allen, 1981). However, for species more widely distributed, the conservation of total genetic diversity of (that) species *in-situ* is difficult. Although species conserved in their natural habitats have the potential for continued evolution of a particular trait within the species and are subject to natural selection, there are indeed many problems in establishing this type of reserve, for example, cost, size and maintenance aspects, political and social issues and the danger of genetic wipe out as a result of natural disasters, fire, etc. In particular, this method of conservation is of significance to the wild relatives of crop plants and a number of other crops, especially tree crops and forest species where there are limitations on the effectiveness of *ex-situ* methods

of conservation. The crops of immediate interest for *in-situ* conservation are the perennials that are vegetatively propagated (Hawkes, 1975) and those with seeds that cannot survive in cold storage (Hawkes, 1982). Wild species maintain their original characteristics best in the habitat to which they are adapted, which necessitates the formation of nature reserves in appropriate climatic, altitudinal and latitudinal zones.

Table 2. Biological factors influencing methods used in the conservation of plant genetic resources.

Biological factors	Preferred conservation methods
Perennial species	*In situ*/ field genebanks/seed and or pollen storage
Annual species	Seed and or pollen storage *in vitro* field genebank
Orthodox species	Seed storage
Recalcitrant seeds	*In vitro/ in situ*/ field gene bank
Synthetic seeds	As orthodox seeds
Vegetatively propagated species with viable seeds	Field genebank pollen *in vitro* cryopreservation
Long living pollen	Pollen storage
Tissue culturing feasibility	If low, look for alternative method
Cryopreservation feasibility	If low, look for alternative method
Genetic stability	If low for certain method, alternative method

Source: *S.D. Shikhamany.*

Ex-situ Conservation

Ex-situ conservation means literally, "off-site conservation". It is the process of protecting a species of plant or animal outside of its natural habitat. It is a conservation strategy that entails the removal of germplasm resources (seed, pollen, sperm, individual organisms), from their original habitat or natural environment, keeping components of biodiversity alive outside of their original habitat or natural environment. Modern *ex-situ* conservation techniques have emerged for saving the genetic biodiversity on our planet and the diversity in their gene pool by guarding against genetic erosion through modern concepts like seedbanks, tissue banks. Cryopreservation techniques are used to freeze these living materials and keep them alive by storing them submerged in liquid nitrogen tanks. Thus, preserved material can then be used to protect diversity in the gene pool.

The *ex-situ* form of conservation includes, in a broad sense, the botanic gardens and storage of seed or vegetative material in genebanks. The field genebanks where clonal materials are maintained as living collections in a field/orchard or plantation also represent *ex-situ* form of conservation. However, field genebanks have the potential risk of germplasm being lost due to disease, stress or disaster, and large amount of space and labor are required to maintain a small proportion of diversity. Cryogenic preservation of vegetative material is another mode of *ex-situ* conservation and it holds promise, especially for base collections.

Efforts to conserve genetic resources *ex-situ* in seed genebanks have accelerated in the past decades. In the genebank, the aim is to provide ideal storage conditions so that the mean viability period of the seeds is greatly extended by reducing the life processes to a low level. Successful seed storage depends on effective control of several factors including

temperature, seed moisture content, storage atmosphere, etc. in response to storage conditions. Seeds within heterogenous germplasm accessions frequently deteriorate at different rates thereby causing selection within the samples to favor genotypes more amenable to given storage conditions. The selection within the germplasm accessions during seed conservation and subsequent regeneration has a strong influence on the genetic composition of an accession. This is one aspect of *ex-situ* conservation in genebanks that makes it desirable to ensure indefinite maintenance of some wild populations of most crops *in-situ. In-situ* conservation has its own set of risks and difficulties but can function as an evolutionary insurance for long-term germplasm availability.

Ex situ conservation of plants involves field gene banks, seed banks and *in vitro* (tissue culture storage). A seed bank is an effective and compact storage for orthodox seeds. They are kept in long-term storage facilities at low temperatures between -20 and -180ºC. Most seeds are expected to remain viable for 20-30 years under medium-term storage and for up to 100 years in long-term storage depending upon the species, the initial seed quality, and infrastructural facility (Koo *et al.*, 2002). Conservation of seed propagated plants is relatively easy for seeds with orthodox type of storage behaviour, i.e., the viability can be maintained by drying the seeds and storing these at low temperature. For orthodox or desiccation tolerant seeds, lower seed moisture content is associated with an increase in storage life of a sample within certain limits. Recalcitrant seeds are relatively short lived (few weeks to months) even under high moisture conditions and require different storage techniques (Stanwood, 1985). *In-vitro* cultures and cryogenic preservation offer promising avenues to overcome the recalcitrant characteristics. However, the genetic stability of *in-vitro* cultures has yet to be fully ascertained before an entire collection is committed to this storage technique. For orthodox seeds, large-scale mechanical refrigeration systems, which hold seeds at temperatures down to -20° C, have greatly increased the storage life of a seed sample, making *ex-situ* conservation of seed germplasm an easy and safe method of conservation. However, deterioration and loss of viability can still occur with increasing time in storage. The longevity of seeds or the maintenance of seed viability is a balance between extrinsic and intrinsic deleterious factors and repair or protective mechanism. Depending on the particular mechanism(s) involved and external factors, such as storage temperature, seed moisture content and oxygen availability, the life span of a seed sample may be shortened or extended.

Limitations in Field and Seed Gene Banks

Field gene bank

In field gene banks the plant genetic resources are kept as live plants that undergo continuous growth and require continuous maintenance. They are often used when the germplasm is either difficult or impossible to conserve as seeds (i.e. when no seeds are formed, seeds are recalcitrant or seed production takes many years, as for many tree species) or the crop is reproduced vegetatively. Field genebanks are mostly used for the conservation of clonal crops which are vegetatively propagated such as potato, sweet potato, yams, cassava, several fruit tree species and many others. The conservation of gene banks is labor intensive and difficult. Field genebanks are generally more expensive to maintain, requiring more labor, more inputs and more space (land) than other methods of conservation. They also have higher levels of risk from natural disasters and adverse environmental conditions like drought, floods or attacks from pests and diseases, to which they are almost continuously exposed (Reeds *et al*., 2004). This can lead to sudden loss of valuable germplasm or accumulation of systemic pathogens, especially viruses. These field

genebanks do not represent the entire range of genetic variability within the respective crop genepool and most of them represent only a fraction of the variability which should be conserved (Withers and Williams, 1985). The occurrence of extreme climatic events (such as El Nino and La Nina that brings about periods of drought,) floods, fires landslides and volcanic eruptions lead to loss of biodiversity. Pest infestation and disease infection are serious threats to field gene banks. Failure to detect and quickly remove diseased sample collections poses a great risk to germplasm conservation.

Seed bank

The conservation of seeds of economically important crops has been practiced since times immemorial and it suffers severe limitations like low seed viability and heterozygosity. Seeds can be maintained for decades or even centuries if the conditions are controlled at <5% humidity and –20°C. Not all species are suited to this treatment. Within the group of orthodox seeds, there is considerable variation between species in length of storage period, which can be achieved under any given set of conditions, varying from comparatively long periods for many of the major cereals through intermediate periods for some of the grain legumes, and relatively short periods for some grasses and several vegetable species. Although a controlled atmosphere is essential for safe storage of seeds, both for long-term and short-term periods, the conditions for long-term storage are more exacting because the seed viability is to be preserved as long as it is possible. The operational cost of storage facility per unit seed stored increases considerably as the requirements of temperature and relative humidity become more stringent. In addition to inherent seed-to-seed variation in any constant storage environment, the actual longevity can be affected by the genotype, various environmental factors that affect seed quality before storage (e.g. ripening, harvesting, drying and processing) and the conditions under which the seeds are stored. Seeds need to be regularly germinated to renew stock or the seeds will eventually loose their viability. Seed banks are at risk from power failure, natural disasters and war. Duplicate stocks can be maintained. Seeds kept in seed banks do not evolve with changes in the environment. For medium storage, the germplasm is stored at 0 to -50^{o}C temperature and 15 to 20% humidity and for long storage at -20 to -180^{o}C.

Importance of Tissue culture

One of the most important conservation methods to have significantly transformed with biotechnological advances is tissue culture (*in vitro* techniques), which have to a large extent addressed the difficulties often associated with field and seed gene banks.

Field and seed bank are not amenable to several problems confronting conservation of plant genetic resources. Some crops do not produce viable seeds while some seeds remain viable for a limited duration only and are recalcitrant to storage. Seeds of certain species deteriorate rapidly due to seed borne pathogen and some seeds are very heterozygous and therefore not suitable for maintaining true to type genotypes. Tissue culture techniques provide an effective approach to circumvent these problems. Plant tissue culture denotes genetically all cell, tissue, and organ cultures. The technique involves separation of cell/tissue/organ from the donor plant under aseptic conditions and growing it on synthetic medium in a suitable container in a controlled environment. It has been successfully applied to germplasm conservation and transfer. It has basically addressed issues related to pests and diseases, quarantine concerns for introduction of diseases from one region to the other, the large space requirements of field gene banks and *in situ* conservation, as well as provision of a relatively simple mechanism of germplasm conservation and transfer. Tissue culture beyond germplasm conservation and transfer

needs have also proved to be very valuable in plant genetic improvement covering somatic embryogenesis, organogenesis, enhanced auxillary buds, callus cultures, *in vitro* mutagenesis, protoplast isolation culture and fusion; *in vitro* flowering, micrografting and genetic transformation. *In vitro* preservation techniques help to conserve germplasm disease free, but also involves lower labor costs and requirement for technical personnel, besides limiting disease-transfer.

The miniaturization of explants reduces space requirements and consequently labor costs for maintenance of germplasm collections. Tissue culture has proved very valuable and necessary in the conservation of *r*ecalcitrant seeds, bulky vegetative material, scarce genetic resources and for immature seeds. It has also been very critical to the distribution and exchange of field gene banks, which are difficult to transfer due to the risk of disease transfer. The several advantages associated with the use of tissue (*in vitro*) culture in germplasm conservation and germplasm transfer are summarized as follows:

- Flexibility – collection at any time, independent of flowering periods for each species (assuming seeds are not required)
- Clonal materials may be produced
- Rapid multiplication
- Potential for virus elimination for contaminated tissue through meristem tip culture
- Germination of difficult immature seed/embryo may be facilitated for breeding
- Distribution across borders may be safer
- Important for collection, multiplication and storage of plant germplasm
- Allow propagating material with high multiplication rates in aseptic environment
- Virus-free plants can be obtained through meristem culture in combination with thermotherapy
- Extend intervals between subculture and other handling operations
- Greater control of production schedules for *in vitro* materials
- Collection, multiplication and storage
- Propagation with high multiplication rate
- Conserve virus free plants through meristem culture
- Reduction in space requirement

Although tissue culture has proved very useful in germplasm conservation and transfer, there are still challenges associated with its use. Some viroids and viruses particularly are not necessarily eliminated or even detected and can readily multiply in tissue culture (Upadhya, 1988). These can be eliminated by meristem or shoot tip cultures possibly in combination with both heat and cold therapy. With *in-vitro* techniques, there has been gradual improvement in the provision of germplasm storage procedures, which uniquely combine the possibilities of disease elimination and rapid clonal propagation (Henshaw and Grout, 1977). Further, the virus-tested cultures could provide ideal material for international exchange and distribution of germplasm as they will be acceptable to plant quarantine authorities (Paroda *et al.,* 1987) and comply with international quarantine regulations.

In Vitro Conservation Techniques

In vitro techniques are effective for the establishment of active and base germplasm especially for plant species that cannot be stored as true seed and are amenable to micropropagation technologies (Fay, 1994). Under *in vitro* conservation, plants may be

stored as normal *in vitro* culture propagation, in reduced growth rate or under suspended growth conditions. Basically, *in vitro* techniques fall into two broad categories: (1) slow growth and (2) cryopreservation (Scowcroft, 1984). Tissue culture can be conserved at either relatively low temperature (15-20^{o}C or cryopreserved in liquid nitrogen at -196^{o}C (no growth). The former is applied to *In vitro* Active Gene Banks (IVAG) or *In vitro* Base Gene banks (IVBG). IVAG is used for short- to medium-term storage while IVBG is applied to long-term storage. Properties for successful conservation include: minimized growth and development of *in vitro* materials to extend sub-culturing intervals; maintenance of viability with minimum risk to genetic stability as well as maintenance of full development and functional plant materials. Explants often used for *in vitro* conservation are mainly axillary buds and meristems. Conditions required by explants for *in vitro* conservation are freedom from competition, nutrients and a highly controlled environment.

Slow growth techniques (Short/medium term conservation)

The basic principle underlying this form of conservation is the induction of reduced vegetative growth of the stored material. It involves normal *in vitro* culture under standard culture conditions (SCC) involving reduced temperature (low temperature for cold tolerant species; higher temperature for tropical species (cold sensitive)), reduced light, media supplements (osmotic inhibitors, growth retardants), tissue dehydration. Growth is limited by modifying culture medium e.g. reduce sugar and/or mineral element concentrates. Medium-term storage is also enhanced by reduction of oxygen levels available to cultures. Medium-term storage is from 1 to 4 years. It supports reduction of growth and increases the intervals between cultures.

The advantage of this approach is that cultures can be readily brought back to normal culture conditions to produce plants on demand. However, the need for frequent sub-culturing may pose a great disadvantage, including contamination of cultures as well as imposition of selection pressure with subsequent change in genetic make-up due to somaclonal variation.

A typical procedure (Grout, 1995) is as follows:

- Prepare culture vessels with appropriate standard medium
- Establish fresh explants from active *in vitro* material growing at optimal temperature
- Incubate the culture to be stored at their optimal temperature (4-10^{o}C)
- Transfer the cultures to their appropriate storage temperature
- Establish a maintenance level light regime with 16-hour photoperiod (at 500-1000 lux being widely accepted)
- Inspect cultures weekly
- At a pre-selected point, cultures are taken from the storage conditions, transferred to the growth medium and returned to optimal growth conditions

Cryopreservation (long-term conservation*)*

Cryopreservation is a process where cells or whole tissues are preserved by cooling to low sub-zero temperatures, such as (typically) 77 K or −196°C (the boiling point of liquid nitrogen). However, when vitrification solutions are not used, the cells being preserved are often damaged due to freezing during the approach to low temperatures or warming to room. Vitrification usually requires the addition of cryoprotectants prior to cooling. Cryoprotective pretreatment procedures involve the reduction in the water content

of the cells prior to freezing (Withers, 1985a). Cryoprotectants can be used either alone or in combination, as no single protectant or combination seems to be the best (Ulrich, 1985). Amongst various cryoprotectants used, mannitol, proline, hydroxyproline, betaine aldehyde, sucrose, sorbitol, methanol, ethylene glycol, dimethyl sulphoxide, glyceraldehyde, glucose and glycerol have been in frequent use. According to some research workers (Ulrich, 1985; Withers, 1985a; Finkle *et al.*, 1985), cryoprotectants reduce the amount of ice at any temperature during freezing and moderate the rise in concentration of solutes, thereby maintaining cell viability.

Cryopreservation is the most promising method of *in-vitro* germplasm storage (Stushnoff and Fear, 1985). Cryopreservation has been successfully applied to callus, protoplast, pollen, meristems, zygotic and somatic embryos and suspension cultures. The first report of successful cryopreservation of plant cell suspension and regeneration of somatic embryos from cryopreserved cells, led to numerous studies on cryopreservation of plant systems (Finkle *et al.*, 1985; Kartha, 1985a; Steponkus, 1985; Withers, 1985a; b).

Cultures are conserved at very low temperature (-196^{o}C) in liquid nitrogen to arrest mitotic and meiotic activities. The procedure ensures long-term preservation of germplasm in genetically unaltered state (Steponkus, 1985). With this method, being relatively convenient and economical, large numbers of genotypes and variants could be conserved which would maximize the potential for storage of genetically desirable material. Most often cryopreservation is used for recalcitrant seed, *in vitro* tissues from vegetatively propagated crops, species with particular gene combinations (elite genotypes), and differentiated plant cells. There is still a limited number of cases where it is used routinely for plant germplasm conservation because the techniques needs to be adapted for each species and this is a function of its natural freezing resistance, explant size and type, and its water content.

The critical phases of Cryopreservation are:

- Storage – Usually in liquid nitrogen (-196ºC) to avoid changes in ice crystals that occur above -100ºC
- Thawing – Usually rapid thawing to avoid damage from ice crystal growth
- Recovery – Thawed out cells must be washed of cryoprotectants and nursed back to normal growth

The key requirements for cryopreservation include: (a) Pre-culturing – usually a rapid growth rate to create cells with small vacuoles and low water content; (b) cryoprotection – to protect against ice damage and alter the form of ice crystals; and (c) freezing – which is the most critical phase. Freezing may be by slow freezing, which allows for cytoplasmic dehydration; or quick freezing, which results in fast intercellular freezing with little dehydration.

Classical cryopreservation techniques comprise cryoprotective treatments, followed by slow freezing in a freezing apparatus. It is based on chemical cryopreservation and freeze induced dehydration of samples during cooling. Usual cryoprotective substances include DMSO (dimethylsulfoxide), mannitol, sorbitol, sucrose and PEG (polyethylene glycol). Cryoprotective substances have principally an osmotic action. Some of them, e.g. DMSO, can enter cells and protect cellular integrity. For most material, optimal freezing conditions consist of a slow cooling rate (0.5-2^{o}C /min) down to around

-40^{o}C. This is followed by immersion of samples in liquid nitrogen. Classical cryopreservation procedures are mainly used for freezing undifferentiated cultures such as cell suspensions and calluses

Alternative new cryopreservation techniques include:

1. Encapsulation–dehydration procedure
   - Explants are encapsulated in alginate beads
   - Pregrown in liquid medium enriched with sucrose for several days
   - Partially dessicated down to a water content of 20%

2. Vitrification procedure
   - Place explants in presence of highly concentrated cryoprotective solution
   - Then freeze rapidly

3.Encapsulation–vitrification procedure
   - Explants are encapsulated in alginate beads
   - Treated with vitrification solutions before freezing

4. Dessication procedure (simplest)
   - Dehydrate explants
   - Freeze rapidly by direct immersion in liquid nitrogen
   - Used mainly for zygotic embryos or embryonic axes extracted from seeds

5. Pre-growth procedure
   - Preculture the plant material on a medium containing cryoprotectants (generally sugars such as glucose or sucrose
   - Dehydrate under the laminar airflow cabinet or with silica gel
   - Then freeze rapidly

6. Droplet freezing procedure
   - After dissection, apices are precultured with DMSO for a few hours
   - Freeze rapidly in droplets of cryoprotective medium placed on aluminium foil

Rapid thawing, which was found suitable for most of the cryopreservation protocols, is accomplished by removing the specimen from liquid nitrogen and immersing them in a warm (34-40°C) water bath for about 1-2 min or until the phase change occurs and ice is transformed into water. Re-growth of cryopreserved specimen is the most accurate criterion for assessing viability, as compared to other methods such as vital staining or using the triphenyl tetrazolium chloride test (Kartha, 1985b; Withers, 1985a; b). Various factors, such as, handling of the specimen, incorporation of certain additives in the re-growth medium and the physical environment during early regrowth are important.

In Vitro Germplasm Transfer

Germplasm exchange *in vitro* has many advantages over conventional methods. It occupies less space and affords the exchange of plant material free of contaminants (pests and diseases). It is of special significance in germplasm transfer. Quarantine provides breeders with the opportunity to access a large number of exotic germplasm of interest. *In vitro* germplasm transfers therefore facilitate easy distribution of plant genetic resources. It

is important to use suitable, impact resistant and well sealed culture containers. The packaging should provide adequate thermal insulation and protection against rough handling. The culture medium should be of a higher than usual concentration of gelling agent. The best shipping method should be used for delivery. The basic steps in tissue culture germplasm transfer involve the following steps:

- Collection (acquisition or exchange)
- Quarantine
- Preparation of plants
- Disease indexing (quarantine)
- Culture initiation
- Micropropagation (copies)
- Characterization
- Storage (standard culture conditions or cryopreservation)
- Distribution

In terms of risk, *in vitro* cultures have the advantage that the mass of material transferred is reduced, non-obscure pests and pathogens are excluded, as is soil, and the transferred material is contained. Without appropriate therapy and indexing, *in vitro* culture alone cannot guarantee a pathogen-free status. *In vitro* exchange has been used for a number of years by institutes, including several International Agricultural Research Centers (IARCs), to distribute germplasm efficiently and securely.

Procedures for the detection of bacterial, fungal and viral pathogens are generally based on symptom detection, whole-plant bioassay, or biochemical or molecular techniques. The application of appropriate therapy techniques also plays an important role in decreasing the risk involved with transfer of material. Aside from the systematic application of therapy techniques and indexing, heat treatment and meristem-tip cultures are also used. Other alternative techniques include shoot-tip grafting, cold treatment, chemotherapy, other physiological/environmental host manipulations (e.g. high carbon dioxide levels).

There has been significant progress in serology. Techniques such as ELISA and serologically specific electron microscopy (SSEM) also called immunosorbent electron microscopy (ISEM), are reliable diagnostic tests for many diseases. The use of monoclonal antibodies is another step towards more specific and more reproducible results. Isolation of dsRNA, electrophoresis of extracted nucleic acid, and NASH techniques have opened new ways for pathogen detection. All of these techniques combined with amplification techniques make it possible to detect extremely low concentrations of viruses and viroids. The development of broad spectrum tests, including detection of dsRNA, monoclonal antibodies to epitopes that are highly conserved between viruses of a given taxon and broad spectrum DNA probes for hybridization assays, are all potentially very useful for general disease indexing purposes (IBPGR, 1988).

The indexing of material held *in vitro* will only be successful if (i) adequate quantities of tissue can be generated for testing, and (ii) adequate concentration of the disease-causing organism develops *in vitro* as a result of the operation of culture conditions. This is the opposite strategy to *in vitro* eradication. Sampling strategies will need to be determined both with respect to the explant selected for *in vitro* inoculation and the selection of material from culture for indexing. There is evidence for both greater and lesser titres of pathogen in culture than in the parent plant; some attenuation may occur *in*

vitro and it should be determined whether this is due to loss of symptom expression or a genuine reduction in titre of pathogen (IBPGR**,** 1988).

In addition to screening using infectious inocula, it may also be possible to utilize *in vitro* reactions to pathogen toxins, thus increasing the safety of indexing. The development of *in vitro* indexing using either infectious or non-infectious inocula would require the establishment of adequately sensitive indicator cultures, themselves certified free of infection by complementary tests (IBGR**,** 1988).

Application in Cassava

Cassava is a clonally propagated crop and originates from Latin America. The International Center for Tropical Agriculture (CIAT) holds the largest cassava germplasm collection with over 6,000 accessions, including landraces, mainly from Latin America, but also about 300 from Asia, as well as some elite clones selected by CIAT and the International Institute of Tropical Agriculture in Nigeria. A subset of these collections described as the "core collection" was assembled and represent the genetic diversity of the complete germplasm collection in a more manageable size. The cassava core collection consists of 630 accessions from the original germplasm bank (Hershey *et al*., 1994). They are being preserved *in vitro* at the Genetic Resources Unit at CIAT. CIAT has over the years maintained the core collection and has distributed these materials in Africa, Asia and Latin American to countries and partners. A duplicate of the complete core collection is being maintained *in vitro* at the Rayong Field Crops Research Center of the Department of Agriculture in Thailand, and is currently being characterized in the field.

The genetic resources held in trust in the genebank of CIAT were assembled with the help of countries which provided the materials on the understanding that it will be made available world-wide. CIAT has since allowed unrestricted access to these useful plant genetic resources in their collections. Slow growth techniques are routinely used to store *in vitro* over 5000 accessions, providing a secure source of healthy plants to compliment the field collections. CIAT has established procedures for management of *in vitro* slow growth conservation of cassava. Cryopreservation protocols have been developed for shoot tips, seeds and embryos. Before conservation, the materials were tested for diseases to facilitate conservation of healthy *in vitro* cultures and to permit sharing of healthy plant cultures with partners or collaborators. The *in vitro* germplasm thus enhances the transfer of good quality germplasm. The accessions have also been largely micro-propagated by *in vitro* techniques to produce large copies which are distributed to partners. The basic procedure used for disease testing and release of plant genetic resources at CIAT are as follows:

- New tips are produced *in vitro*; harvested; transferred to new media and treated by thermotherapy (+37^{o}C day/35^{0}C night) for 12 days.
- Newly developed shoot tips go through a total of 3 cycles of thermotherapy; after which they are grown under normal conditions at +26-28ºC.
- The plants developed *in vitro* are tested for cassava virus diseases using ELISA.
- If one plant from one shoot shows presence of the virus all plants are destroyed.
- Virus tested plants are transplanted into sterilized soil and are re-tested for frog skin disease by grafting to a healthy hypersensitive clone and analysis by RT-PCR (**Figure 1**). Plants that are negative in all tests are released.

One of the key tests used at CIAT is the ELISA test (**Table 3**). Elisa has been used effectively for virus and pathogen detection in plants. Each virus has a unique protein

coating, hence they can be detected by using antibodies, which are unlinked to enzymes for reporting the affinity binding via color signal from the used enzyme substrate. The double antibody sandwich (DAS) method in a 96-well micro-titre plate (Clark and Adams, 1977) is still widely used. Various alternative formats, substrates and antibody binding modifications have been developed over the years to increase specific sensitivity (Agdia, 2002; Bioreba, 2002). However, ELISA has several limitations. The test is specific only to a given strain of the pathogen. For a large group of ubiquitous viruses, which do not produce disease symptoms or fatal consequences, ELISA kits have not been developed. Thus, the strain specificity has severe limitations for comprehensive quality control. Further, ELISA is less sensitive than PCR, and may fail to detect low amounts of the virus in tissue culture plants. A positive ELISA is a good indicator of the existence of microbes (but false positives may also occur), and a negative test does not strictly ensure their absence (Schmidt *et al.,* 2004). The Immuno-Tissue-Printing technique allows the localization of viruses and is therefore an improvement of the elimination strategies *in vitro* by visualizing the result of virus removal (Fitch *et al*., 2001).

Due to limitations of the ELISA, tests are also conducted using the PCR method. The PCR is more sensitive than ELISA and can detect pathogens in extremely low amounts. PCR detects pathogens from DNA or RNA. This technique uses a specific enzyme and the respective genomic start code for a relevant section of the pathogen-DNA to reproduce millions of copies from it. If the target DNA concentration is low, then the sample could test negative, and the plant material could be regarded as free of the target microbe. A negative test often is more reliable of practical freeness of the pathogen from the plant material, and this technique reduces the number of false negatives (i.e. materials which test negative) even though they carry the virus. PCR has the advantage that by determining 'degenerate primers", a large group of viruses, bacteria and fungi can be detected (Schmidt *et al*., 2004).

Table 3. Seed health testing methods applied at CIAT for cassava to detect bacteria (B), fungi (F), viruses (V), nematodes (N), insects (I) and weeds (W).

Seed health test	Cassava
Seedling symptom test	F,B,V
Blotter test	F
Agar test	F
Indicator test	V
Washing test	F
Immuno enzymatic test (ELISA)	V
Dilution plating test	B
Direct visual inspection	F,N,I,W
Polymerase Chain Reaction (PCR)	V

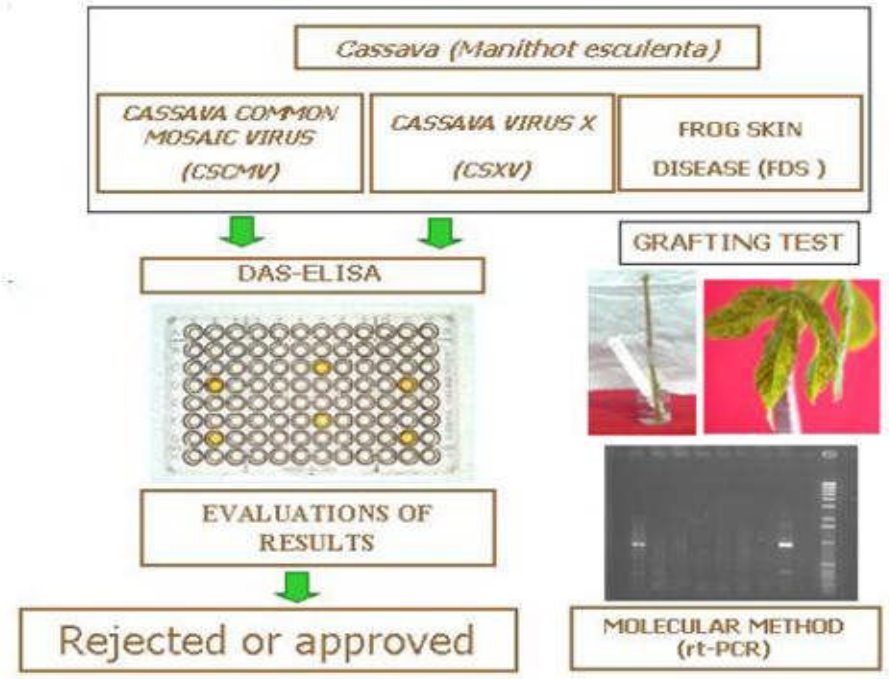

Figure 1. Detection of viruses.
***Source**: GRU, CIAT*

The process for the transfer of germplasm at CIAT involves the following steps:

- Receipt and handling of institutional requests
- Multiplication of disease-free clones
- Evaluation, packing and shipment of cultures

Figure 2 shows the transfer process from the originating institute (CIAT) to the recipient partner, while the comprehensive flow chart of the operations involved are shown in **Figure 3**. The distribution of cassava by CIAT's Genetic Resources Unit (GRU) is given in **Figure 4.** The partners receiving plant genetic resources from CIAT include the national agriculture research institutes (NARs), universities, commercial companies, and regional organizations. About 65% of the germplasm released has been utilized by the different CIAT research teams, while the remaining 35% where used by external institutions and users.

CONCLUSIONS

There has been significant advancement in research demonstrating increasing applications of *in-vitro* techniques and cryopreservation technologies in the genetic conservation of germplasm, particularly of vegetatively propagated crop plants and other difficult materials. *In-vitro* techniques now provide suitable approaches, which can lead to the safe conservation of germplasm employing slow growth procedures. Long-term preservation of meristems (shoot tips) is also on the increase. Cryopreservation of seeds, pollen, excised embryos/embryonic axes and buds has also proved feasible and practical in

many cases. Aside of it being an efficient means for germplasm conservation and transfer, it has proven to be a good strategy for maintaining good quality plant materials, in addition to efficiently minimizing the risk of genetic erosion associated with field gene banks.

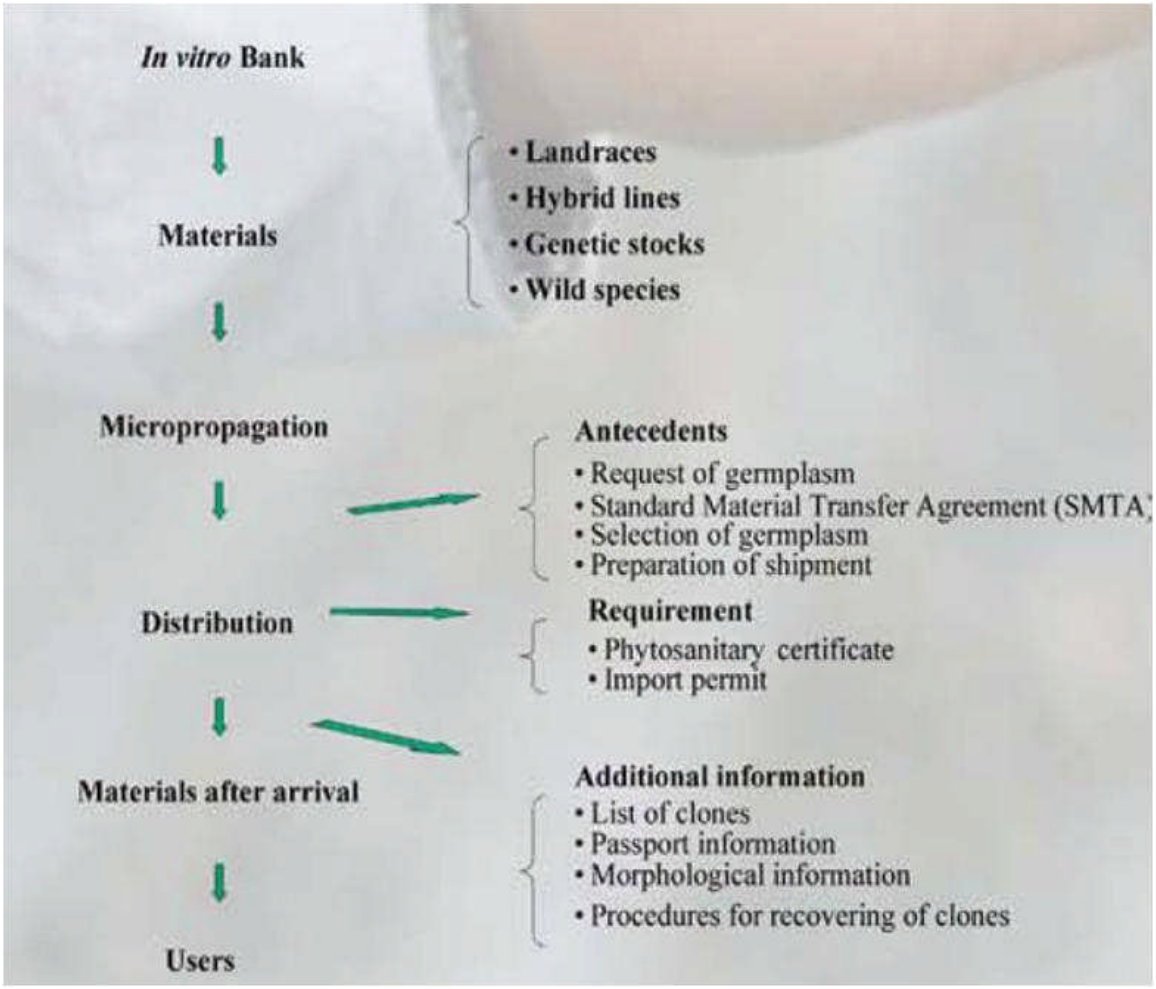

Figure 2. Flow of activities for the transfer of plant genetic resources at CIAT.
***Source**: GRU, CIAT.*

The potential of plant tissue culture in increasing agricultural production has now been well recognized by both investors and policy makers. FAO has long perceived plant tissue culture as a main technology for the developing countries for the production of disease-free, high-quality planting material and its commercial applications. However, in many developing countries, the establishment cost of the facilities is high. Many international organizations, including FAO (FAO, 1993), agree that tissue culture technology is very relevant to agriculture, provided the high cost of production is satisfactorily resolved. Plant tissue culture techniques have a vast potential to produce plants of superior quality, but this potential has not been fully exploited in the developing countries.

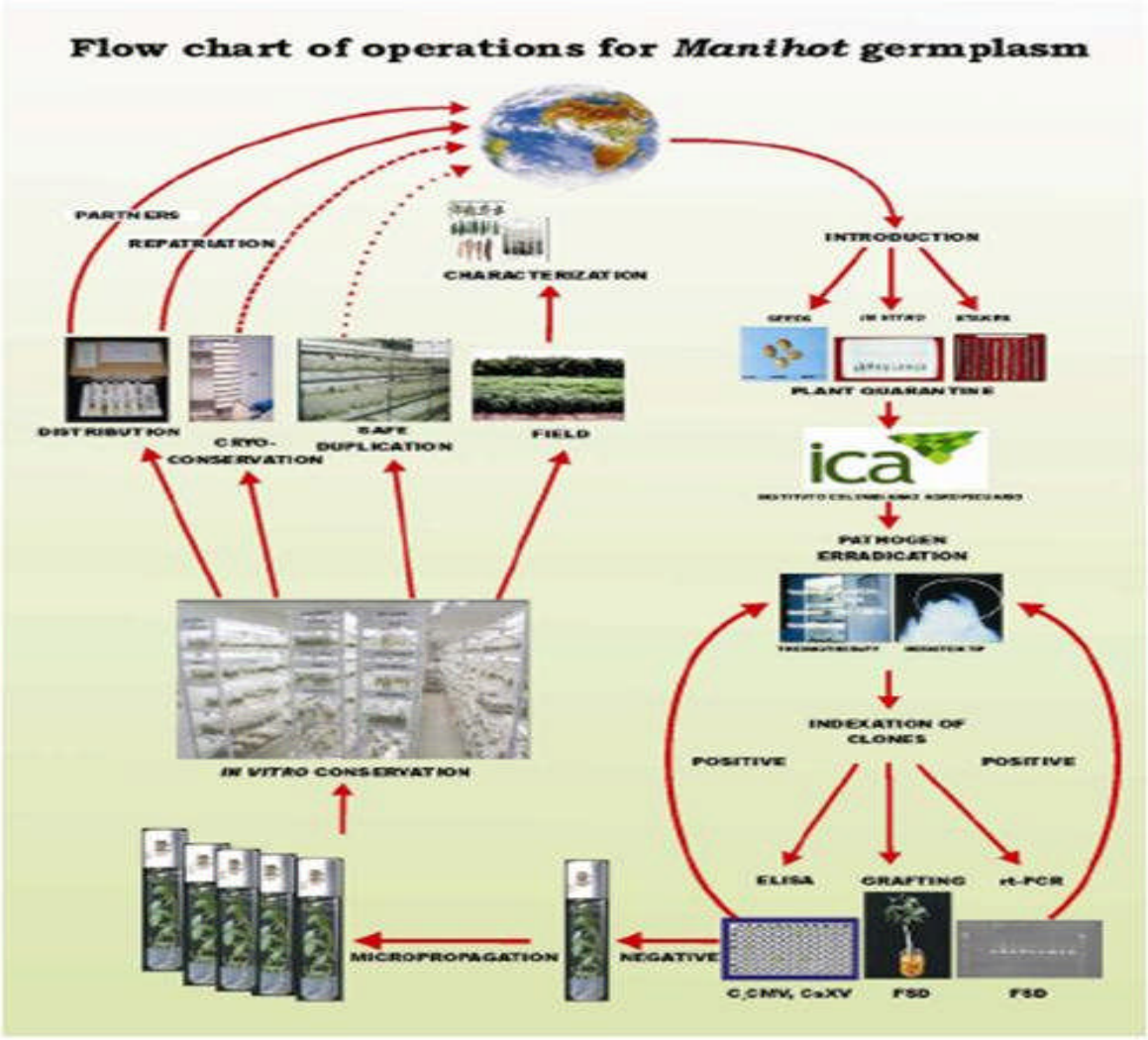

Figure 3. Flow chart of operations for Manihot germplasm at CIAT.
***Source**: Mafla et al., 2008.*

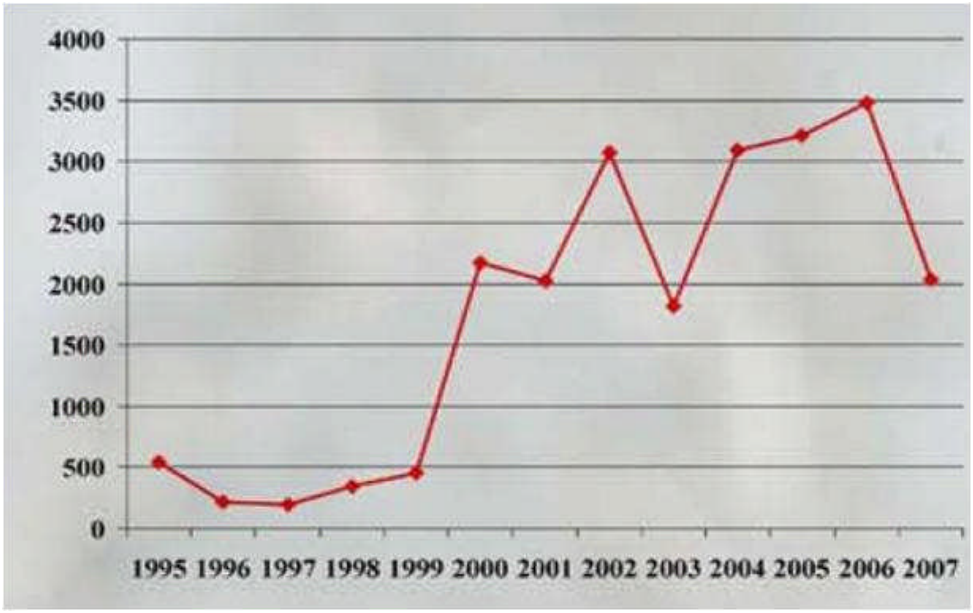

Figure 4. Distribution of cassava germplasm by CIAT's Genetic Resources Unit between 1995 and 2007.

Many problems beset the management of *in vitro* conservation in developing countries, foremost of which is funding. The lack of full government and institutional financial support results in incomplete fragmented efforts in conservation of plant genetic resources. Inadequate funding affects the entire management spectrum, from personnel, facilities, to number of accessions. Lack of well-equipped cold storage facilities for short-, medium- and long-term *in vitro* storage and propagation of germplasm in poor countries have tended to be restricted only to those plant materials identified as having key potential use.

Low-cost tissue culture techniques provide an efficient means for cutting costs and to make this technology widely available in resource-poor institutes or organizations. Low-cost tissue culture techniques is the adoption of practices and use of equipment to reduce cost. A number of low-cost alternatives can be used to simplify various operations and reduce the costs in a tissue culture facility. Careful planning of a tissue culture facility can make large savings, both in the construction costs and in day-to-day operations in the facility. Proper choice of media and containers can reduce the cost of micro-propagation. It is envisaged that as cost cutting measures are introduced for *in vitro* techniques, the technology will go a long way to achieving its wide-spread maximization and application in germplasm conservation and transfer for thousands of plant species grown by mankind.

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Appendix 1 (Source: Roca *et al.*,1984).

Preparation of the Culture Medium

A) Basal Medium. Can be prepared in either of two forms:

1. Using stock solutions of mineral salts, vitamins and growth regulators. (For the preparation of the Murashige and Skoog stock solutions, see Appendix 1a).
   To prepare 1 liter of medium, to 500 ml of double distilled water add:

   20.0 ml of stock solution No. 1
   1.0 ml of stock solution No. 2
   1.0 ml of stock solution No. 3
   2.9 ml of stock solution No. 4
   5.0 ml of stock solution No. 5

2. Using the pre-made Murashige and Skoog medium in powder form (without sucrose and without vitamins and agar). Each bag contains 4.3 g of powder, which serves to prepare 1 liter of basal medium. The powder can be stored at 8-10^{o}C, under dessication, for up to 2 years. To prepare the basal medium, dissolve the entire contents of one bag in 500 ml double distilled water. Add the same volumes of stock solutions No. 1 through No. 5 as in 1, above.

B) Supplements. Once either of the basal media is ready, proceed as follows:

- Add 5.0ml of stock solution No. 6 and 6.25 ml of stock solution No.7
- Dissolve 20.0g of sucrose
- Add 5.0 ml of the benzyl aminopurine stock solution (see Appendix 1b); 5.0 ml of the gibberellic acid stock solution (see Appendix 1b); 2.0 ml of naphathalene acetic acid stock solution (see Appendix 1b)
- Complete to 700 ml with double distilled water
- Adjust the pH to 5.7-5.8
- Dissolve by heating 6.0 g of agar in 300 ml of double distilled water.
- Mix well the medium with the agar solution.

C) Sterilization. Quickly distribute the prepared medium in 18-X. 150-mm test tubes (5 ml/tube); let cool slightly and cap the tubes.
Autocave the tubes with the mwdium; 15 pounds (121^{o}C) per square inch during 15 minutes; decompress slowly.
Place the tubes in afresh place until the agar is solid , then store them in darkness at 6-8oC until used.

Appendix 1a. Preparation of Murashige and Skoog Stock Solutions

To prepare the stock solutions, dissolve, one by one , all the ingredients presented in Table 1, in the volumes of double distilled water shown.

Appendix 1b. Preparation of Growth Regulator Stock Solutions

Benzyl aminopurine (10 ppm): Dissolve 20 mg in a small volumes of 1.0 N HCl; complete to 200 ml with double distilled water (this is a 100-ppm solution of the hormone): take 20 ml of the 100-ppm solution and complete to 200 ml (this is the 10-ppm stock solution).

Gibberellic acid (10 ppm): Dissolve 22 mg (90% gibberellic acid) in a small volume of 1.0 N KOH; complete to 200 ml with water, take 20 ml of this solution and complete to 200 ml with water.

Naphathalene acetic acid (10 ppm): Dissolve 20 mg in a small

Murashige and Skoog stock and medium preparation

Stock solution No. a	Constituents Substance	Amount	Volume of stock per 1 liter basal medium
1	NH_4NO_3	82.5 g	20.0 ml
	KNO_3	95.0 g	
	$MgSO_4 . 7H_2O$	18.5 g	
	KH_2PO_4	8.5 g	
	Dissolve in 1000 ml water		
2	H_3BO_3	0.62 g	1.0ml
	$MnSo_4 . H_2O$	2.176 g	
	$ZnSO_4 . 7H_2O$	0.86 g	
	$Na_2MoO_4 . 2H_2O$	0.025 g	
	$CuSO_4 . 5H_2O$	0.0025 g	
	$CoCl_2 . 6H_2O$	0.0025	
	Dissolve in 100 ml water.		
3	Kl	0.075 g	1.0 ml
	Dissolve in 100 ml water.		
4	$CaCl_2 . 2H_2O$	15 g	2.9 ml
	Dissolve in 100 ml water.		
5[b]	a) Na_2EDTA	1.492 mg	5.0 ml
	b) $FeSO_4 . 7H_2O$	1.114 mg	
	Dissolve in 200 ml water.		
6	Thiamine-HCl	10 mg	5.0 ml
	Dissolve in 100 ml water.		
7	m-inositol	0.8 g	6.25 ml
	Dissolve in 100 ml water.		

a. Stocks 2 and 6 should be kept frozen; all the others at 8-10ºC. Keep stock 5 protected from light.

b. Separately dissolve a and b in 50 ml water each; heat up b in a water bath; mix both solutions well; let cool and then add water to complete to 200 ml.

Volume of 1.0 N KOH; complete to 200 ml with water; take 20 ml of this solution and complete to 200 ml with water.

Addition of Growth Regulator Stocks to the Medium: To determine the volume (Appendix 1) of each growth regulator stock solution necessary to obtain the prescribed concentrations (step B), apply the following formulation:

$C_1V_1 = C_2V_2$
C_1 = Concentration of stock = 10 mg/l
C_2 = Final concentration of growth regulator in the medium:
Benzyl aminopurine = 0.05 mg/l
Gibberellic acid = 0.05 mg/l
Naphathalene acetic acid =0.02 mg/l
V_1 = Volume (in ml) of stock solutions needed = **x**
V_2 = Final volume of medium = 1000 ml

$$X = \frac{0.05 \text{ mg/l} \times 1000 \text{ ml}}{10 \text{ mg/l}} = 5.0 \text{ ml of either benzyl aminopurine or gibberellic acid}$$

$$X = \frac{0.02 \text{ mg/l} \times 1000 \text{ ml}}{10 \text{ mg/l}} = 2.0 \text{ ml of naphathalene acetic acid}$$

Appendix 2

Equipment Checklist
The needs for equipment vary widely with the type of culture system used and the capacity of the facility.

Preparation room
Autoclave
Water distillation
Double sink unit
Hot plate with magnetic stirrer
pH meter
Weighing balance (1 - 200g)
Weighing balance (1.0 - .0001g)
Oven for drying glassware
Microwave oven
Refrigerator
Freezer
Trolley for carrying hot media flasks and containers
Cupboards along the walls for storage of chemicals

Transfer room
Laminar flow cabinets
Bench with presses for storage of containers with media
Peristaltic pump for pouring medium
Height adjustable chairs
Safety burners
Binocular microscope
Gyratory shaker
Inverted microscope
Compound microscope
Shaker for low speed use

Growth room
Shelving unit frames
Artifical lighting
Airconditioning

CHAPTER 9

THEORY AND USE OF MOLECULAR MARKERS

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INTRODUCTION

Genetics is a study of genes that underlie observable phenotypes, and through inheritance studies provide an understanding of the relation between the phenotype and the genotype (Liu, 1998). When dealing with a qualitative character, the relation between the phenotype and the genotype of the parents is easily recognised from simple numerical proportions observed in the segregating progeny (van Eck, 1995). Quantitative traits cannot be described in discrete phenotypic classes, but are described through the trait values of individuals, which are conceived as samples drawn from a continuous distribution (Falconer, 1981). The relation between the phenotypic value and the genotype for most quantitative traits remains obscure with common unanswered questions (van Eck, 1995), such as: How many genes influence the trait? How much does each gene contribute to the trait? Is there additive or non-additive interaction between alleles at the same locus, or epistatic interaction between loci?

Genetic markers, defined as differences at the genotype (DNA) level, can be used to answer and explain questions (Paterson *et al.*, 1991b). To be useful as a genetic marker, the marker locus has to show experimentally detectable variation among individuals in the test population (Liu, 1998). The variation can be considered at different biological levels, from the simple heritable phenotype to detection of variation at the single nucleotide level. Once the variation is identified, and the genotypes of all individuals in the test population are known, the frequency of recombination events between loci can be used to estimate linkage distances between markers. Genetic markers can also be used to study the diversity of the observable variation at the population or species level (Lee, 1995). A genetic marker may therefore be operationally described as a heritable polymorphic marker with clear genetic interpretation and repeatability (Fatokun *et al.*, 1997)**.**

The genetic interpretation of a marker strongly depends on the sequence complexity of the genome and the kind of variation the marker identifies (Liu, 1998). Differences between genotypes of two individuals can be detected in several ways. This could be by casual glance of the individuals at the phenotypic level (visible markers), by an assay of enzymes from tissues, and by analysis of DNA (using DNA markers) (Paterson *et al*., 1991b). Visible markers or macromutations in genes with visible consequences have been used in genetic studies since the early part of the twentieth century (Morgan, 1911).

Until the advent of DNA markers, the genetic markers used to develop maps in plants have been those affecting morphological traits (Liu, 1998). Although these morphological markers are of value, their usefulness in mapping studies (Ellis, 1994) is limited by their paucity and nature because they can be influenced by environmental factors. The number of useful morphological markers for quantitative traits was so limited that in most studies only a few markers were used, representing only a small fraction of the genome (Liu, 1998). However, genetic maps based on morphological markers have been

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developed and a large number of these have been described for some crop species (Ellis, 1994; Tanksley, 1994).

The discovery that allelic forms of enzymes (isozymes or allozymes) can be separated on electrophoretic gels and detected with histochemical activity stains heralded the era of the use of molecular markers in genetic research (Smithies, 1955; Hunter and Markert, 1957). Enzyme coding genes could be screened for polymorphism in natural populations and mapped genetically using electrophoretic techniques independent of any phenotypic change (Lewontin and Hubby, 1966). By the early 1980s, isozyme markers were being employed as a general tool for mapping polygenes. These studies met with considerable success compared to previous studies using morphological markers (Tanksley *et al*., 1982; Vallejos and Tanksley, 1983; Edwards *et al*., 1987; Weller *et al*., 1988). There has been a great deal of progress in the application of isozyme analysis in plant breeding (Weeden, 1989). The genome coverage situation improved with isozyme markers, but the number of available enzyme activity stains limited the number of markers (Liu, 1998). Consequently, informative isozyme markers were not enough to cover an entire genome (Tanksley *et al*., 1982; Vallejos and Tanksley, 1983; Edwards *et al.*, 1987). However the paucity of isozyme loci and the fact that they are subject to post-translational modifications often restrict their utility (Staub *et al.*, 1996).

The next major advance in the utilization of molecular markers occurred with the development of DNA-based genetic markers (Lee, 1995). Botstein *et al*. (1980) suggested that large numbers of genetic markers might be found by studying differences in the DNA molecule. In principle, visible markers and isozymes are as useful as DNA markers. In practice, however, much greater numbers of DNA markers can be readily found. Crop plants have about 10^8-10^9 nucleotides of DNA in total (Paterson *et al*., 1991b). Even if a small percentage of these is different between two individuals, an enormous number of potential DNA markers result. In contrast, relatively few visible markers or isozymes tend to be polymorphic between two randomly chosen individuals (Staub *et al*., 1996; Stuber, 1994).

The level of polymorphism maintained at any given locus in natural populations is determined by many factors, which include population size, mating habits, selection, mutation rate, and migration (Tanksley, 1993). Two of these factors, i.e. relaxed selection pressure and higher mutation rates, cause allelic variation to be higher at the molecular level loci than at morphological marker loci.

The availability of complete genome maps, facilitated by DNA markers, opened the opportunity for new statistical approaches, such as interval analysis for detecting polygenes (Tanksley, 1993). Alleles of most molecular markers are co-dominant, whereas morphological marker loci segregate dominant-recessive alleles (Tanksley, 1993). Thus, the advent of molecular markers has allowed polygene mapping in virtually any segregating population, e.g., F_2, F_3, backcross, and recombinant inbreds (Liu, 1998). Because molecular marker loci do not normally exhibit epistatic or pleiotropic effects, a virtually limitless number of segregating markers can be used in a single population for mapping polygenes across an entire genome (Tanksley, 1993).

DNA sequence variations can be monitored using several techniques. One technique monitors variation as changes in the length of DNA fragments produced by restriction endonucleases. This method has, therefore, been termed restriction fragment length polymorphisms (RFLPs) (Groodzicker *et al*., 1974; Botstein *et al*., 1980). At present, many types of molecular markers with different useful properties have emerged and can be utilized for genetic analysis (Rafalski and Tingey, 1993; Mohan *et al*., 1997). These markers provide an unlimited opportunity to obtain detailed information about

genetic variation in the nuclear genome at the DNA level. The dominant, epistatic or heterotic interactions between alleles from one or more loci can be estimated (Fatokun *et al*., 1992; Stuber *et al*., 1992). The shift from genetics based on the inference of genotype from phenotype, as pioneered by Mendel, to genetics based on the direct analysis of DNA sequence variation has been hailed as an important genetic paradigm shift. Genetic maps have been constructed in many crop plants using these markers on a single segregating population (Mohan *et al*., 1997). While molecular markers have been used extensively, both in crops and in the livestock industry, this chapter highlights mainly the use of markers in crop genetic improvement.

Characteristics of Markers Systems

Various types of molecular markers are utilized to evaluate DNA polymorphism and are generally classified as hybridization-based markers and polymerase chain reaction (PCR)-based markers. In the former, DNA profiles are visualized by hybridizing the restriction enzyme-digested DNA, to a labelled probe, which is a DNA fragment of known origin or sequence. PCR-based markers involve *in vitro* amplification of particular DNA sequences or loci, with the help of specifically or arbitrarily chosen oligonucleotide sequences (primers) and a thermostable DNA polymerase enzyme. The amplified fragments are separated electrophoretically and banding patterns are detected by different methods, such as staining and autoradiography. PCR is a versatile technique invented during the mid-1980s. Ever since thermostable DNA polymerase was introduced in 1988, the use of PCR in research has increased tremendously. The primer sequences are chosen to allow base-specific binding to the template in reverse orientation. PCR is extremely sensitive and operates at a very high speed. Its application for diverse purposes has opened up a multitude of new possibilities in the field of molecular biology. The following are the desirable characteristics of marker systems.

- Highly polymorphic nature
- Codominant inheritance (determination of homozygous and heterozygous states of diploid organisms)
- Frequent occurrence in genome
- Selective neutral behaviour (the DNA sequences of any organism are neutral to environmental conditions or management practices)
- Easy access (availability)
- Easy and fast assay
- High reproducibility
- Easy exchange of data between laboratories.

It is extremely difficult to find a molecular marker which would meet all the above criteria. Depending on the type of study to be undertaken, an appropriate marker system that meets a few of these characteristics would have to be identified.

Molecular Markers (Molecular Marker Technologies)

Several molecular markers exist and the features of the different types of the markers are described in **Table 1**. The most common markers applied to cassava to date are briefly described below.

Restriction fragment length polymorphism (RFLP)

Among the various molecular markers developed, RFLPs were the first to be used in human genome mapping (Botstein *et al*., 1980) and later they were adopted for plant

genome mapping (Weber and Helentjaris, 1989). RFLPs are co-dominant and can identify a unique locus (Tanksley *et al*., 1989). This technique arose from the discovery of restriction enzymes and natural variation in DNA base sequence of organisms (Beckmann and Soller, 1986). Restriction enzymes are enzymes that bind specifically to and cut (or modify) double stranded DNA at short, specific sites within or adjacent to a particular sequence known as the recognition sequence (Botstein *et al*., 1980). These enzymes have been classified into three groups, on the basis of their functions, as Type I, Type II, and Type III restriction enzymes. Types I and III carry modification (methylation) and ATP dependent cleavage activities on the same enzyme. Neither Type I nor III are widely used in RFLP analysis. Type II restriction enzymes, which are able to cleave DNA at a specific base sequence (restriction site) are the widely used enzymes in RFLP applications (Kochert, 1990). Recognition sites for various enzymes vary from four to eight base pairs length. Base changes in DNA can alter the sequences that are recognised by restriction enzymes, abolishing sites or creating new sites for particular enzymes (Beckmann and Soller, 1983). This creates an enormous variation in eukaryotic cells. This variation has been exploited with the advent of restriction enzymes, which by nature of their recognition, binding and cleavage properties, reduce large segments of DNA to a series of small fragments of distinct sizes (Kochert, 1990). The number of fragments produced, reflect the distribution of restriction enzyme recognition sites in the DNA.

Table 1. Key features of common molecular marker technologies.

Marker type	PCR based	Uses Restric-tion enzymes	Poly-morphism	Abun-dance	Co-domi-nant	Auto-mation	Lost per assay	Specialized equipment
RFLP	no	yes	moderate	moderate	yes	no	1 to few	Radioactive isotope
RAPD	yes	no	moderate	moderate	no	yes	many	Agarose gels
AFLP	yes	no	moderate	moderate	no	yes	many	Polyacrylamide gels/capillary
ISSR	yes	no	moderate	moderate	no	yes	many	Agarose/ Polyacrylamide gels
DArT	yes	yes	moderate	moderate	no	yes	many	Microarray
CAPS	yes	yes	variable	moderate	yes	yes	single	Agarose gels
SCAR	yes	no	low	moderate	yes	yes	single	Agarose gels
SSR	yes	no	low	moderate	yes	yes	1 to about 20	Polyacrylamide gels/capillary
TE - Anchor	yes	no	variable	variable	yes	yes	single	Agarosegels
SNP	yes	no	variable	highest	yes	yes	1 to thousands	variable

Using RFLP markers, genetic maps have been developed for many plant species (Mohan *et al*., 1997). Restriction enzyme digests of relatively small genomes, such as chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA) produce 40-60 fragments (Maniatis *et al*., 1982). Based on the DNA-DNA hybridization, a piece of radioactively or

chemically labelled chromosomal DNA fragment (probe) is used to detect polymorphism by hybridization to specific fragments in the separated digestion mixture that possess some nucleotide sequence homologous to the probe (Botstein *et al*., 1980). In practice, difficulties can arise if the probe used hybridizes to repeated sequences at multiple locations on the genome (Liu, 1998). In these cases, allelic and non-allelic variation cannot be distinguished. These problems commonly occur when the same probe is used to detect RFLPs in progeny of different lineages. Therefore, it is important to use probes that detect single polymorphic loci in different pedigrees (Liu, 1998**)**. Causse *et al.* (1994) developed a rice genetic map using 800 RFLPs. However, RFLP analysis is labor-intensive and time consuming. The newer approaches based on polymerase chain reaction (PCR) are relatively simple. PCR is a DNA synthesis technique that amplifies specific regions of DNA that lie between two sites defined by the complementary sequences of two specific primers (Liu, 1998).

Random amplified polymorphic DNA (RAPD)

RAPD analysis, a PCR-based molecular marker technique, was developed independently by Welsh and McClelland (1990) and Williams *et al*. (1990). Since then many new modifications of the PCR-based molecular marker techniques have been developed. RAPD markers are generated by PCR amplification of random genomic DNA segments with single-synthetic decamer primers of arbitrary sequence (Williams *et al*., 1990). Amplified products are separated by electrophoresis on agarose or polyacrilamide gels. Polymorphisms are detected as DNA fragments, which amplify in one indvidual but not the other, i.e. present/absent. These changes include most probably single base substitutions as well as deletions or insertions that either change the primer sequence or the size of the amplified DNA (Williams *et al*., 1990).

RAPD markers have many advantages over other methods (Kesseli *et al*., 1992; Williams *et al*., 1990). They can identify large numbers of genetic polymorphisms between closely related taxa, and a large set of primers can be screened within a short period. It requires the use of minimal amounts of DNA, thus allowing simple and rapid methods for genomic DNA isolation. The technique is simple and straightforward, requiring no isolation of cloned probes or preparation of hybridization filters. The presence or absence of a band of a particular size generally distinguishes different alleles at the same locus. The band-present phenotype is dominant to the band-absent phenotype. The band present phenotype may represent a homozygous or heterozygous genotype for the locus in question (Liu, 1998). The band absent phenotype can only represent a homozygous genotype for the alternate allele. A disadvantage is that a test is needed to distinguish between the heterozygotes and the homozygotes.

As the PCR amplification process is dependent upon many components and their interactions (Devos and Gale, 1992; Caetano-Anolles and Bassam, 1993; Wolf *et al*., 1993), it is important to specify a set of reaction conditions in order to obtain reproducible results for a given species. Sources of reliability lie in the purity of the template DNA, magnesium (Mg^{2+}) concentration, the choice of thermal-stable DNA polymerase and thermal cycler used in PCR amplification. It also depends on the imprecise matches between short oligonucleotide primers (decamers) and the template DNA at the low annealing temperatures (35-40^{o}C) of amplification conditions (Iqbal and Rayburn, 1994; Kelly, 1995; Qiu *et al*., 1995).

In practice, optimization for primer choice, PCR conditions and gel reading are needed to obtain RAPD markers with simple genetic interpretation and high repeatability (Williams *et al*., 1990). Since a single primer may generate several polymorphic markers,

screening a large number of primers on a small number of genotypes in a mapping population is a useful method to obtain a large group of markers having high information content (Liu, 1998).

Efforts to overcome problems of reproducibility with RAPD markers led to the development of sequenced-characterized amplified regions (SCARs) (Kesseli *et al*., 1992) and allele-specific associated primers (Weeden *et al*., 1992). Reproducibility is increased by sequencing the two ends of the RAPD fragment and synthesizing two long primers (24 base pairs) homologous to each end. These two primers, which include the original decamer sequence, are used in the PCR protocol at an elevated annealing temperature (50-65^{o}C), and generally produce a single fragment (SCAR) of the same size as the previously sequenced RAPD fragment (Kesseli *et al*., 1992). Paran and Michelmore (1993) and Nair *et al*. (1995, 1996) were able to increase the reliability of RAPD markers by converting them to SCARs, which could be used in a PCR reaction to amplify the RAPD fragments. The SCARs have the advantage of being inherited in a codominant fashion in contrast to RAPDs which are inherited in a dominant manner (Mohan *et al.,* 1997).

Amplified fragment length polymorphism (AFLP)

Amplified fragment length polymorphism (AFLP) is based on PCR amplification of restriction fragments generated by specific restriction enzymes and selective oligonucleotide primers (Vos *et al*., 1995). The genomic DNA is digested with two restriction enzymes, usually a rare cutter and a frequent cutter. Double stranded oligonucleotides, known as adapters are ligated to the ends of the genomic DNA at the specific restriction sites. Adapters have a nucleotide known as a "sticky end", complementary to that of the restriction site. Separate adapters are needed for each of the different restriction enzymes. The ligated DNA is then used as a template for PCR reactions. The primers are specific to the combination of the adapter sequence. The AFLP method generates a large number of restriction fragment bands, which is then selectively reduced by primers that have one or 3 different nucleotides at the 3' prime end, facilitating the detection of polymorphisms. Choosing different base numbers and composition of nucleotides in adapters can control the number of DNA fragments, which are amplified. The PCR products are separated on denaturing polyacrylamide gels. Caution is needed in scoring the AFLP gel because of the large number of bands. The AFLP bands are usually scored as dominant markers, but occasionally polymorphisms can be distinguished as codominant markers (Liu, 1998). To do this, a mixture distribution model can be used to fit the band intensity for three possible genotypes such as in a di-allelic model. This approach is very useful in saturation mapping and for discrimination between varieties. Lin *et al*. (1996) compared three different DNA mapping techniques i.e. RFLP, RAPD, and AFLP, for efficiency in detecting polymorphism in soybean and found AFLP to be the most efficient technique. High reproducibility, rapid generation and high frequency of identifiable polymorphisms make AFLP analysis an attractive technique for identifying polymorphisms and for determining linkages by analyzing individuals from a segregating population.

Minisatellites (VNTR) and Microsatellites (SSR)

PCR with specific primers can only reveal polymorphisms that lie in the amplified area between the primers (Ubi, 1998). An alternative approach to increase the utility of PCR-based markers is to produce primers that flank genomic regions more likely to show variability than a randomly selected sequence (Kochert, 1994). Such hypervariable regions consist of tandem repeated DNA sequences. Markers based on such sequences include

minisatellites and microsatellites. Minisatellites are tandem repeats of sequences ranging from 9 to 100 bp in the genome (Liu, 1998). The number of the repeats varies and is usually less than 1000. Minisatellites are also referred to as Variable Number of Tandem Repeats (VNTR) and are detected mainly by hybridization approaches (Liu, 1998). In hybridization, genomic DNA can be digested using restriction sites flanking the tandem repeats. The cutting yields fragments containing cores of the repeats with different number of repeats (length variation). The polymorphic bands result from the variation in the number of the tandem repeats.

Microsatellites or simple sequence repeats (SSR) are tandem repeats of a much smaller size (2-8 bp) and ubiquitous in eukaryotes (Gianfranceschi *et al*., 1998). SSR polymorphism (SSRP) reflects polymorphism based on the number of repeat units (Litt and Luty, 1989; Weber and May, 1989; Arunachalam and Chandrashekaran, 1994). They are highly variable DNA sequences that can be used as informative markers for the genetic analysis of plants and animals. A genetic map with over 6000 SSRs has been constructed in mouse (Dietrich *et al*., 1996). The number and composition of microsatellite repeats differ in plants and animals. The frequency of repeats longer than 20 bp has been estimated to occur every 33 kb in plants, unlike mammals where it has been found to occur every 6 kb (Wang *et al*., 1994). The more common form of repeats are simple di-nucleotide repeats such as (CA)n, (GT)n, (GA)n:(CT)n, (CG)n:(GC)n, and (AT)n:(TA)n, where n is the number of repeats. In humans, AC or TC is a very common repeat unit, but in plants AT is more common, followed by AG or TC (Powell *et al*., 1996). In general plants have about 10 times fewer SSRs than humans.

Microsatellites with tri- and tetra-nucleotide repeats are also found, but their frequencies are lower than the di-nucleotide repeats (Hearne *et al*., 1992). Searching through DNA sequence databases for sequences containing simple repeats may help identify microsatellites (Liu, 1998). For some species, such as human, mouse, *Arabidopsis* and rice, a large amount of DNA sequence has already been accumulated. The discovery, inheritance and variability of fourteen GA repeats have been described for cassava (Chavarriaga-Aguirre *et al*., 1998). A subset of those SSR markers were used to evaluate the genetic diversity of the core collection of about 600 accessions of the cassava world germplasm bank at the International Center of Tropical Agriculture (CIAT) (Chavarriaga-Aguirre *et al*., 1999). The development and characterization of 172 SSR primers in cassava have also been reported (Mba *et al*., 2001).

Primers are designed to flank repeats found in sequence databases (Mba *et al*., 2001). Nucleotide sequence flanking the repeats is used to design primers to amplify the different number of repeats in different varieties. This type of polymorphism is highly reproducible. These primers are very useful for rapid and accurate detection of polymorphic loci and the information could be used for developing a high-density genetic map based on these sequence tags (Schmidt and Heslop-Harrison, 1996; Roder *et al*., 1998). For most plant and animal species where no sequence data are available, a large effort using hybridization and sequencing is needed to identify microsatellites suitable for use as genetic markers (Liu, 1998). Hybridization using simple repeats as probes is used to screen genomic clones, to identify a clone containing the sequenced microsatellites. The clone is then sequenced and primers designed from sequences flanking the repeats. Microsatellite markers have proven to be one of the most effective tools for genetic mapping marker-assisted breeding and diversity studies. With new techniques for enriching and pre-screening libraries, it is now possible to produce greater numbers of microsatellite markers (Edwards *et al*., 1996; Mba *et al*., 2001).

Single-strand conformation polymorphism (SSCP)

When the objective for using markers is the detection of mutations involving a single nucleotide change, then a method that detects changes in a nucleotide sequence for an entire fragment of more than 1000 bp, such as single-strand conformation polymorphism (SSCP) will be appropriate (Liu, 1998). SSCP is a technique that can detect polymorphism and can detect DNA sequence alterations as small as a single nucleotide change (Orita *et al*., 1989). It is a powerful and rapid method, but it can only be used with relatively short DNA fragments. However, SSCP can identify the heterozygosity of the DNA fragment in DNAs of same molecule weight. Electrophoretic mobility of single-stranded DNA in non-denaturing polyacrylamide gels depends on both size and nucleotide composition. This method exploits the tendency of single-stranded DNA to form intra-molecular base pairs, resulting in a sequence dependent conformation with a specific mobility in acrylamide gels. Changes in DNA sequence, even in a single base pair, can cause alterations in the conformation and result in changes in electrophoretic mobility. In practice, SSCPs is principally detected by PCR to amplify a specific fragment, which is then run on a conformational gel (high-resolution acrylamide gel).

Sequence tagged sites (STS)

Sequence tagged sites (STSs) were proposed by Olson *et al.* (1989) as chromosome landmarks in the human genome. A STS is a short unique fragment of DNA whose sequence and position in the genome are known (~300 bp) (Liu, 1998). Large DNA clones contain the same STS overlap, so STSs can be used in physical mapping to order large DNA fragments (Liu, 1998). If a polymorphism can be detected using STS as probe, then anchor points between genetic and physical maps can be established (Weissenbach *et al*., 1992; Gyapay *et al*., 1994). The polymorphic STS markers are also commonly used for genomic analysis in plants (Mazur and Tingey, 1995).

Expressed sequence tags (EST)

Expressed sequence tags (ESTs) are subsets of STSs derived from cDNA clones (Liu, 1998). ESTs can serve the same purpose as the random STSs, with the advantage that ESTs are derived from expressed genes, i.e., from spliced mRNA which is usually free of introns as well as repetitive DNA. ESTs have the advantages of representing functional genes and are therefore more useful as genetic markers than anonymous non-functional sequences (Liu, 1998). In species having large genomes, cDNA sequencing to obtain ESTs are advantageous for genome analysis. In cassava, EST has been developed from transcipt-derived fragments (TDFs), which are AFLP fragments of expressed mRNA population. Suarez *et al*. (2000) obtained more than 500 TDFs by applying cDNA-AFLP techniques to mRNA from parents of a cassava genetic mapping population. Sequence alignment of the ESTs revealed mostly genes of unknown function. Generation of ESTs as differentially expressed sequences, in time or between different varieties, is an important way of developing ESTs around specific traits for the candidate locus approach to mapping complex traits (Boventius and Weller, 1994).

Sequence characterized amplified regions for amplification of specific band (SCAR)

Michelmore *et al.*(1991) introduced this technique wherein the RAPD marker termini are sequenced and longer primers are designed (22-24 nucleotide bases long) for specific amplification of a particular locus. These are similar to STS markers in construction and application. The presence or absence of the band indicates variation in

sequence. These are better reproducible than RAPDs. SCARs are usually dominant markers; however, some of them can be converted into codominant markers by digesting them with tetra cutting restriction enzymes and polymorphism can be deduced by either denaturing gel electrophoresis or SSCP (Rafalski and Tingey, 1993). Compared to arbitrary primers, SCARs exhibit several advantages in mapping studies (codominant SCARs are more informative for genetic mapping than dominant RAPDs), map-based cloning as they can be used to screen pooled genomic libraries by PCR, physical mapping, locus specificity, etc. SCARs also allow comparative mapping or homology studies among related species, thus making it an extremely adaptable concept in the near future.

Cleaved amplified polymorphic sequences (CAPs)

These polymorphic patterns are generated by restriction enzyme digestion of PCR products. Such digests are compared for their differential migration during electrophoresis (Koniecyzn and Ausubel, 1993; Jarvis *et al*., 1994). PCR primer for this process can be synthesized based on the sequence information available in databank of genomic or cDNA sequences or cloned RAPD bands. These markers are codominant in nature.

Inter simple sequence repeat markers (ISSR)

In this technique, reported by Zietkiewicz *et al.* (1994), primers based on microsatellites are utilized to amplify inter-SSR DNA sequences. Here, various microsatellites anchored at the 3' end are used for amplifying genomic DNA, which increases their specificity. These are mostly dominant markers, though occasionally a few of them exhibit codominance. An unlimited number of primers can be synthesized for various combinations of di-, tri-, tetra- and penta-nucleotides [4^3=64; 4^4=256] etc. with an anchor made up of a few bases, and can be exploited for a broad range of applications in plant species.

***Single nucleotide polymorphisms (SNPs*)**

SNPs are a new set of molecular markers being used in genetic studies. They are an abundant source of sequence variants and of all the molecular marker technologies available today, they provide the greatest marker density (Edwards and McCough, 2007). A single-nucleotide polymorphism (SNP, pronounced *snip*) is a DNA sequence variation occurring when a single nucleotide (A, T, C, or G) in the genome (or other shared sequence) differs between members of a biological species or paired chromosomes in an individual. For example, two sequenced DNA fragments from different individuals, AAGC**C**TA to AAGC**T**TA, contain a difference in a single nucleotide. In this case, we say that there are two *alleles*: C and T. Almost all common SNPs have only two alleles. Within a population, SNPs can be assigned a minor allele frequency (the lowest allele frequency) at a locus that is observed in a particular population. This is simply the lesser of the two allele frequencies for single-nucleotide polymorphisms. The benefits of SNP assays include increased speed of genotyping, lower cost and the parallel assays of multiple SNPs (Edwards & McCough, 2007). SNPs are indispensable in such applications as association mapping and construction of high-density genetic maps, which usually require genotyping of thousands of SNPs in a large number of individuals (Akhunov *et al*., 2009). Single nucleotide polymorphisms (SNPs) are ideally suited for the construction of high-resolution genetic maps, studying population evolutionary history and performing genome-wide association mapping experiments. Two main advantages of SNPs over other molecular markers are in terms of their abundance (Zhu *et al*., 2003) and availability of a wide array of technologies for high throughput SNP analysis (Fan *et al*., 2006).

Microarray

A DNA microarray (also commonly known as gene chip, DNA chip, or biochip) is a collection of unique DNA probes that are arranged in a regular lattice on a solid surface. In standard microarrays, the probes are synthesized and then attached via surface engineering to a solid surface by a covalent bond to a chemical matrix (via epoxy-silane, amino-silane, lysine, polyacrylamide or others). The solid surface can be glass or a silicon chip, in which case they are colloquially known as an *Affy chip* when an Affymetrix chip is used. Each probe contains DNA microspot (10^{-12} moles) and is composed of a DNA sequence which could be a short section of a gene or other DNA element that are used to hybridize a cDNA or cRNA sample (called *target*) under high-stringency conditions. The probes are therefore complementary to the sequence of interest. Nucleic acid "targets" are applied to these probes in a hybridization fluid. Targets will anneal to complementary probes and unhybridized target is washed away. Probe-target hybridization is usually detected and quantified by detection of fluorophore-, silver-, or chemiluminescence-labeled targets to determine relative abundance of nucleic acid sequences in the target. DNA microarrays can be used to measure changes in expression levels, to detect single nucleotide polymorphisms (SNPs), or to genotype or resequence mutant genomes.

DNA arrays are different from other types of microarray only in that they either measure DNA or use DNA as part of its detection system. Other microarray platforms, such as Illumina, use microscopic beads, instead of the large solid support. Alternatively, microarrays can be constructed by the direct synthesis of oligonucleotide probes on solid surfaces. Microarrays also differ in fabrication, workings, accuracy, efficiency, and cost. Several comprehensive reviews cover different microarray platforms and approaches [Sevenet and Cussenot, 2003; Hardiman, 2004; Stoughton, 2005; Ahmed, 2006a,b; Kawasaki, 2006]. Probe choices for microarrays may include amplified cDNA clones, PCR gene products, or different lengths of oligonucleotides (Kawasaki, 2006).

Studies examining the correlation among microarray technologies have focused primarily on differences between probe types (Yauk and Berndt, 2007). However, many other factors contribute to technical variability. Methods of printing/deposition of probes onto glass slides include contact-spotting using pins, deposition by ink jet, or *in situ* synthesis of oligonucleotides on the slide (Hughes *et al*., 2001; Gao *et al*., 2004). Slide surfaces may be coated with different types of matrices that govern the affinity of probe binding and affect background fluorescence (Sobek *et al.*, 2006). Target preparation varies and may include different amounts of starting RNA, amplification, and labeling methods (Gold *et al*., 2004; Hardiman, 2004; Schindler *et al*., 2005; Singh *et al*., 2005; Kawasaki, 2006), all of which contribute to the type and quality of data produced. In addition, cDNA and several oligonucleotide platforms allow experiments to be carried out in one or two colors (Patterson *et al*., 2006). Two color experiments may involve dye-swap, reference RNA, or loop designs (Patterson *et al.*, 2006).

Hybridization can be undertaken manually or using automated hybridization stations; optimization of methods is important to minimize array variability and hybridization artifacts (Yauk *et al*., 2005; Han *et al*., 2006; Yauk *et al.*, 2006). The scanner (high or low laser powers) and scanner settings influence background fluorescence, the number of saturated spots and the number of spots below background (Shi *et al.*, 2005b; Timlin, 2006), and should be adjusted to maximize the linear dynamic range. Acquisition of data from images can be carried out using various algorithms through different commercial packages. The final critical steps include applying the appropriate filtering methods, evaluating microarray data quality (Shi *et al*., 2004), normalization (Bilban *et al*., 2002b; Quackenbush, 2002), and data analysis (Shi *et al*., 2005a; Jeffery *et al*., 2006).

Normalization and detection of differential gene expression are key to ensuring the accuracy and reproducibility of data across time, laboratories, and platforms, and are reviewed in detail elsewhere (Bilban *et al*., 2002b; Quackenbush, 2002; Armstrong and van de Wiel, 2004; Reimers, 2005; Breitling, 2006).

Choosing a Molecular Marker Technology

Marker assisted breeding offers significant time savings when making genetic advances that are otherwise difficult to evaluate or manipulate (Burr *et al*., 1983; Tanksley *et al*., 1989). Choosing the right molecular marker system is critical to success and implementation cost. Integrating markers into breeding can potentially reduce the expense, time and efforts needed for marker-assisted breeding (MAB). The factors include marker type, amenability to simple technology, polymorphism, reproducibility of results, and if markers are mapped. PCR-based markers are relatively easier to use and require less DNA. Markers such as SSR, SNP, RAPD require less DNA. The level of polymorphism is also very important in increasing the power to detect QTL (QTL mapping) and for efficient use of QTLs in MAB. SNPs and SSRs are the most polymorphic and choice markers.

The critical considerations for appropriate markers are reliability, DNA quantity and quality, technical application, polymorphism and cost (Mackill and Ni, 2000). Markers should be tightly linked to target loci at preferably less than 5cM genetic distance. The use of flanking markers or intragenic markers will greatly increase the reliability of the markers. Some marker techniques require large amounts and high quality DNA, which may be difficult to obtain in practice. The level of simplicity of marker techniques is also crucial to the successful utilization of the procedure. High throughput, simple and quick methods are highly desirable. Ideally, the marker should be highly polymorphic and the marker assay must be cost effective in order for MAS to be feasible. The most widely used markers are SSR. Sequence tagged site (STS), sequence characterized amplified region (SCAR) or single nucleotide polymorphism (SNP) markers that are derived from specific DNA sequences of markers (e.g. RFLP) and are linked to a gene or quantitative trait locus(QTL) are also extremely useful for marker-assisted selection (MAS) (Sharp *et al*., 2001).

Applications of Molecular Markers in Plant Genome Analysis and Breeding

Molecular markers have been looked upon as tools for a large number of applications ranging from localization of a gene to improvement of plant varieties by marker-assisted selection. They have also become extremely popular markers for phylogenetic analysis adding new dimensions to the evolutionary theories. If we look at the history of the development of these markers, it is evident that they have been improved over the last two decades to provide easy, fast and automated assistance to scientists and breeders. Genome analysis based on molecular markers has generated a vast amount of information and a number of databases are being generated to preserve and popularize it.

Molecular markers can be used to construct high density maps (i.e. maps with many markers of known locations, interspersed at relatively short intervals throughout the genome), which provides the framework needed for the application of marker-assisted selection (MAS). Using the map, and other marker-traits association strategies, such as the QTL mapping and bulk segregant analysis, putative genes can then be detected by testing for statistical association between the markers and traits of interest (**Figure 1**). This could be for simple traits such as those for disease resistance (cassava mosaic disease) or

morphological traits (leaf shape in cassava) controlled by one or few genes. The traits could also be genetically complex quantitative characters involving many genes (i.e. QTL) and environmental effects. Most of the economically important traits such as yield and yield components belong to this latter group. Having identified markers linked to traits (either located beside or within genes of interest), such markers can be used in breeding.

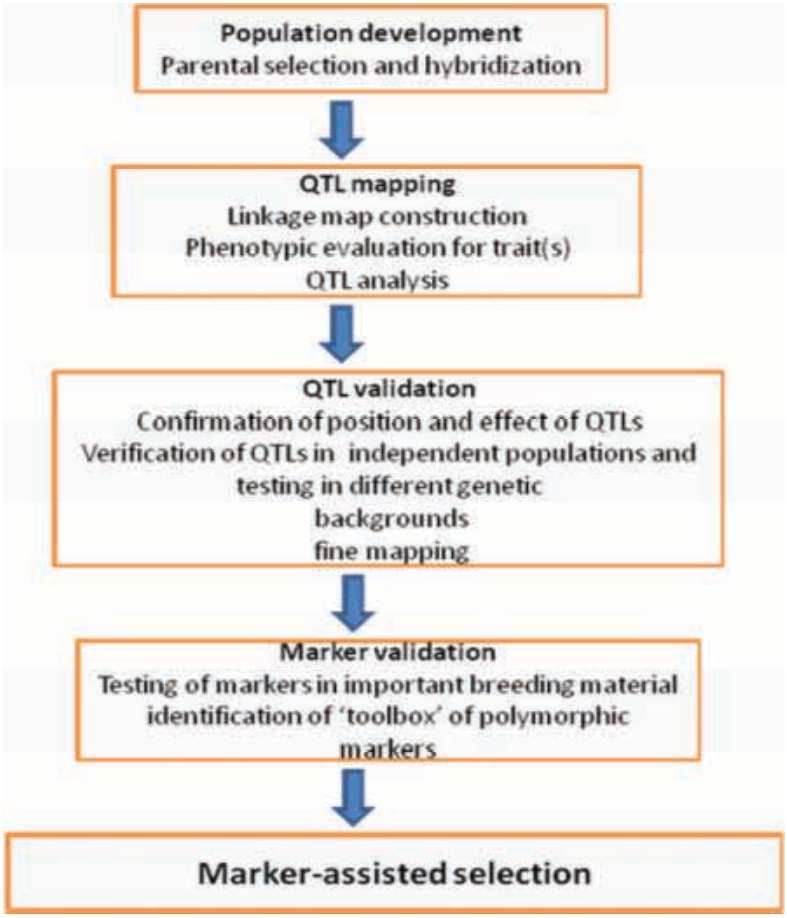

Figure 1. Scheme showing utilization of molecular markers in gene discovery and application in molecular breeding.
***Source**: Collard and Mackill, 2008.*

Assessing and managing genetic diversity

Crop genetic diversity is the engine that drives plant improvement programs (Lee, 1995). Knowledge of genetic diversity, the useful variability and relationships among sets of germplasm, is beneficial to sustained crop improvement (Paterson *et al*., 1991b). Historically, inferences have been based on reproductive biology, eco-geographic data, morphology, ontogeny, pedigree records, breeding behaviour, *in situ* and *ex situ* evaluation

of agricultural traits, chromosome structure and behaviour, and protein markers, among others (Lee, 1995). Each perspective has provided valuable information. However, a limitation of the above methods was providing an accurate estimate of variability as measured by heterozygosity.

The prospect of utilizing DNA marker technology for assessing and managing genetic collections has been widely reviewed (Kresovich and McFerson, 1992; Bretting and Widrlechner, 1995). Surveys of germplasm collections with DNA markers have revealed ecogeographical distributional patterns of "genetic" variation that could be used to develop sampling strategies for curators and breeders of annual (Lubbers *et al*., 1991; Goffreda *et al.*, 1992; Kresovich *et al.*, 1992) and perennial crop species (Besse *et al.*, 1994). With increased availability of DNA sequence data, and information on gene functions, it is now possible to conduct molecular assessments of diversity among large samples of germplasm and relate it to productivity on an enhanced trait (Lee, 1995).

Assessments of genetic diversity of elite crop germplasm have been sought and used by plant breeders for numerous reasons, such as genetic relationships, parent selection, germplasm management, and germplasm protection, among others (Lee, 1995). DNA markers provide superior discriminatory power relative to protein and morphological markers (Smith and Smith, 1992). Several studies have evaluated and compared estimates of genetic diversity based on the coancestry coefficient *(f)* and DNA markers. The studies demonstrate that DNA markers provide a more accurate portrayal of genetic diversity among sets of elite germplasm (Messmer *et al.*, 1993; Nienhuis *et al*., 1992; McGrath and Quiros, 1992; Siedler *et al*., 1994; Graner *et al*., 1994; Gerdes and Tracy, 1994). In general, genetic distances measured based on DNA markers and *f* have been positively correlated, thus placing entries into the same general groupings. Even when pedigree records have been acceptable indicators of genetic relationships, DNA-based estimates have provided additional useful information (Smith *et al., 1990*). DNA markers have represented a significant improvement in the plant breeder's perception of genetic diversity. Compared to the various methods available for detecting DNA polymorphism, DNA markers provide a comprehensive coverage of the genome, and have become a standard tool for assessing genetic diversity (Lee, 1995). Molecular markers have also been used to study relationships among cassava accessions and their wild relatives (Bertram, 1993; Ocampo *et al*., 1995, Roa *et al*., 1997) as well as in quantitative assessment of genetic similarity in cassava (Beeching *et al*., 1993; Second *et al*., 1997; Elias *et al*., 2000).

Selection of parents for source populations and hybrid combinations

Methods of parent selection may be considered under two broad categories, *a priori* (direct evaluation of the parents) and *a posteriori* (involving progeny testing) (Baenziger and Paterson, 1992). Plant breeding programs of annual crops have relied predominantly on the latter category, especially in the development of F_1 hybrid cultivars (Lee, 1995). Experienced breeders with core germplasm have used a *priori* methods more commonly for simply inherited traits (Lee, 1995).

Production of hybrid cultivars has been a goal of many crop-breeding programs convinced of the merits of heterosis, uniformity and the economics of seed production (Fehr, 1984). Decades of selection in many crops have produced a highly productive germplasm base, and their pedigree records may be helpful in estimating genetic distances based on alleles that are identical by descent (Lee, 1995). These resources, if supplemented with DNA marker data, could help breeders develop crossing schemes of maximum efficiency. This could be achieved by avoiding crosses between closely related parents and by focusing on crosses likely to yield hybrid progeny having the desired degree of

heterozygosity (Paterson *et al*., 1991b). Several studies using RFLP-based estimates of genetic similarity among elite maize inbreds have demonstrated the utililty of DNA markers for placing lines into their respective heterotic groups (Lee *et al*., 1989; Melchinger *et al*., 1991; Dudley *et al*., 1991; Livini *et al*., 1992; Messmer *et al*., 1993).

An implicit purpose for establishing and using heterotic groups has been the desire to predict the performance of hybrids created by intergroup crosses (Paterson *et al*., 1991b). Such ability is needed because *per se*, performance of parents has not been sufficiently correlated with the performance of their hybrid progeny for important traits (Lee, 1995). However, markers can provide additional useful information and guidance for the development of hybrid cultivars for crops lacking well-established heterotic groups (Lee, 1995).

Evolutionary relationships and comparative mapping

An important use of DNA markers has been the attempt to elucidate evolutionary relationships, within and between species, genera, or larger taxonomic groupings. Such studies involve studying similarities and differences among taxa, using numerous genetic markers (Paterson *et al*., 1991b).

Although phylogenetic trees have previously been established for many species on the basis of isozyme markers and chromosome homology (Rick, 1979; Riley, 1965; Beasley, 1942; Kimber, 1961; Philips, 1962), DNA markers have recently added to the breadth of phylogenetic information available (Song *et al*., 1988; 1990; Galau *et al*., 1988; Debener *et al*., 1990; Miller and Tanksley, 1991). Such studies are important in classifying newly discovered germplasm, and in establishing possible sources from which valuable traits might readily be transferred to crop species.

In a few cases, it has been possible to reveal the consequences of evolutionary divergence on chromosome organization in crop species. For example, the chromosomes of tomato are remarkably similar to those of potato. Based on the mapping of 134 common markers in the two species, seven chromosomes showed no detectable rearrangement, and the remaining five showed a total of seven paracentric inversions (Bonierbale *et al*., 1988). Pepper and tomato, which are more distantly related, retain homology to many common cDNA probes, but differ by a larger number of arrangements (Tanksley *et al*., 1988). The consequences of evolutionary divergence have much practical value. By defining the sites of chromosomal rearrangement, one also defines intervening regions in which genes are arranged similarly in different organisms. This might permit extrapolating results from tomato to pepper (Tanksley *et al*., 1988). Thus, exhaustive studies of tomato may help to fill in the gaps left by less detailed studies of pepper using the technique of comparative mapping. Recognition of the considerable conservation of these features within sets of plants such as rice, wheat, and maize (Ahn *et al*., 1993); sorghum and maize (Pereira *et al*., 1994); tomato, potato and pepper (Tanksley *et al*., 1988; 1992); and *Arabidopsis* and Brassica (Teutonico and Osborn, 1994) has led to the suggestion of considering such groups as single genetic systems (Helentjaris, 1993; Bennetzen and Freeling, 1993).

Comparative mapping is also possible for QTLs. Comparative mapping with DNA clones has provided the basis for investigations of gene position across species. For example, a genome region that conditions the absence of ligules is observed in rice, wheat, and maize (Ahn *et al*., 1993). Similar inspections of the linkage data of other taxa, has revealed many other examples, such as the conserved position and order between genes for resistance to leaf rust (*Puccina* spp.) and prolamines in oats, wheat, and maize (Rayapati *et al*., 1994a; b) which are all grasses. This pattern of conserved linkage and function has been extended to include quantitative trait loci (QTLs). The initial report of orthologous

QTLs noted that the RFLP loci with the greatest effects on seed weight in mungbean and cowpea were detected by the same RFLP clones (Fatokun *et al*., 1992).

Paterson *et al*. (1991a) compared the locations of QTLs in two different species of tomato, (*Lycopersicon chmielewskii* and *L. cheesmanii*, which are distantly related (Rick, 1979; Miller and Tanksley, 1991). Both are similar in having very small fruits with highly soluble solids. About half of the QTLs mapped in the two species fell at similar chromosomal locations, suggesting that the same genetic factors influence some quantitative traits in the two distantly related species. Thus, QTL mapping information from one pedigree might be somewhat predictive of QTL locations in other pedigrees or other related species.

Often, the genome size of one member of the group is many-fold smaller than other members. The smaller genome size would accelerate positional cloning of orthologous genes (Paterson *et al.*, 1991b; Lee, 1995, Liu, 1998). Once the gene in the source species has been cloned and sequenced, this information may be used to isolate the orthologous gene in the target species. This has been demonstrated by the isolation of the gene for chalcone flavonne isomerase in maize using sequence information from *Petunia*, snapdragon, and bean (Grotewold and Peterson, 1994). Comparisons of locus order and distribution of recombination events may also elucidate barriers and suggest strategies to incorporate germplasm in wide crosses (Devos *et al*., 1993).

Map-based cloning

Targeted isolation of plant genes based strictly on its map position has been strengthened substantially by the advent of DNA markers (Paterson *et al.*, 1991b, Lee, 1995). Genetic maps based on DNA markers have improved the efficiency of established approaches, such as positional cloning, and transposon tagging (Briggs and Beavis, 1994). Positional cloning with yeast artificial chromosomes (YACs) was first used successfully in plants in the model species, *Arabidopsis* (Arondel *et al*., 1992) and subsequently in tomato to isolate genes for disease resistance (Martin *et al*., 1993).

Transposon tagging as a means of gene isolation in plants was first demonstrated in maize (Fedoroff *et al*., 1984). Subsequently, maize transposable element systems were modified and introduced into other plant species to facilitate gene tagging and isolation (Ellis *et al.*, 1988). For example, if elements of the *Ac-Ds* transposon system behave similarly in the new species, one may enhance the chances of tagging a locus by monitoring genetic linkage between it and the transposon. Mapped DNA markers from transposable elements loci have been especially useful for gene cloning in maize because DNA probes of the elements often lead to several fragments of which one is the interrupted gene. The markers help discriminate between linked and unlinked fragments and identify those inserted into the target locus. In maize, examples include *opaque-2* and *Hm1* (Johal and Briggs, 1992), and *rf2* (Schnable and Wise, 1994).

In plants with relatively small genomes, DNA markers, large-scale DNA cloning, and production of transgenic plants have made it possible to clone genes in a relatively short time (Lee, 1995). Together, these and other approaches to gene identification and isolation will help elucidate some of the genetic complexities of important traits and create new opportunities for their manipulation and utilization in plant breeding strategies.

Identifying and introgressing exotic germplasm

Not only do molecular markers provide an unprecedented glimpse into the quantity of genetic diversity; they also provide an opportunity to assess the potential of genes from exotic germplasm once they are in an elite line background (Lee, 1995). Analysis of

advanced backcrosses involving wild relatives with DNA markers indicate that exotic donor parents contribute more genes with positive effects than could have been predicted from their phenotypes alone. This has been shown in maize (Lee *et al*., 1990), tomato (de Vincente and Tanksley, 1993; Eshed and Zamir, 1994) and wheat (Schwarzbacher *et al*., 1992).

Exotic germplasm is an important source of major gene resistance to abiotic and biotic stresses and some quality traits (Vaughan, 1989). Introgression of such genes is enhanced through marker-assisted selection and via an efficient introgression of the genome region without excessive linkage drag (Lee, 1995). In contrast, the role of exotic germplasm in improving quantitative traits has been less prominent. With the advent of DNA markers it has been suggested that it may be possible to develop efficient strategies for rapidly identifying and incorporating favorable exotic alleles into elite backgrounds to realise a net improvement in trait performance. This has been proved in several crops (Edwards, 1992).

DNA markers could also increase the efficiency of germplasm conversion programs such as those used for sorghum (Duncan *et al*., 1991). The goal of the conversion programs for sorghum is to adapt tropical germplasm such that it may be grown and evaluated in temperate regions. Once the adapted growth habit has been achieved, the merit of the exotic genes may be assessed in breeding programs. Such conversion programs might utilize DNA markers at several stages. Selection of exotic parents should promote maximum diversity while minimizing duplications (Lee, 1995). DNA markers could assist with the selection of exotic parents for conversion. When segregating progeny are selected for backcrossing, markers could be used to identify progeny that carry the derived genome region with minimal amounts of the donor parent genome (Pereira and Lee, 1995). This identification would reduce the number of backcross generations and facilitate maximum recovery of exotic alleles. Thus, breeders would have more opportunities for assessing the merits of truly exotic alleles with unique and favorable effects (Lee, 1995).

Analysis of complex traits

Most important agronomic characters are controlled by many genes (Zhuang *et a*l., 1997). However, the number of genes and their interaction are generally poorly understood. There have been attempts to utilize DNA markers to elucidate genetic aspects of quantitative inheritance patterns such as heterosis, epistasis, and the genetic basis of response to artificial selection, for numerous traits and crops (Lee, 1995). While the techniques of quantitative genetics have proved useful in the study of quantitative traits, these characters continue to be more difficult to manipulate in breeding programs than single gene traits (Tanksley *et al*., 1989).

Resolving complex traits into their single gene components will offer the possibility of treating these characters with the efficiency of single gene traits. Thoday (1961) pointed out that the study of quantitative variation is hampered because of the lack of complete genetic maps, a limitation which has largely been overcome with the advent of DNA markers (Botstein *et al*., 1980). Higher density molecular maps make it possible to identify and measure the effects of genes underlying quantitative traits (Tanksley *et al*., 1989; Paterson *et al*., 1991b; McCouch and Doerge, 1995).

QTL analysis provides a way of selectively manipulating individual genetic components of a complex trait. Cytogenetic markers have been used to locate QTLs for several decades in crops such as maize and wheat. However, the advantages of DNA markers, such as improved resolution, coverage, and codominance, make them a better

method for characterization of genomes. In marker–assisted selection (MAS) for breeding and genetics, 15-20 cM is a practical limit of resolution (Lee, 1995). Smaller regions (1-5 cM), is ultimately necessary for maximum efficiency according to simulated MAS (Gimelfarb and Lande, 1994). Molecular markers linked to quantitative traits have been reported for many crop species (Lee, 1995; Lin *et al*., 1996; Zhuang *et al*., 1997; Mohan *et al*., 1997).

Gene pyramiding

Pyramiding is the process of combining several genes together into a single phenotype (Collard and Mackill, 2010). Pyramiding may be possible in conventional breeding, but is usually not easy to identify the plants containing more than one gene. The most widespread application for pyramiding has been for multiple disease resistance genes (i.e. combining qualitative resistance genes together into single genotype.

Response to selection

The genetic basis of response to artificial selection has been a subject of interest (Barton, 1990) leading to many questions. To what extent is observed response attributable to extant genetic variation? What mechanisms are capable of generating genetic variations beneficial to crop improvement programs? What mechanisms stabilize the content and expression of plant genomes? Answers to these questions have important implications for many phases of plant breeding, such as effective population sizes, selection response models, and production of transgenic crops. Slowly, DNA markers are revealing some features of the underlying mechanism (Lee, 1995). In long-term recurrent selection programs high frequencies of fixed RFLP alleles have been found in advanced generations (Sughroue and Rocheford, 1994).

Analysis of selection programs with DNA markers has provided important clues about the transfer and maintenance of genes in plant breeding. Analysis of the Illinois maize long-term selection program found a QTL for low levels of endosperm starch associated with a transposon insertion of the *Sh2* locus (Goldman *et al*., 1993; Alrefai *et al*., 1994). This suggests that transposon could be an important source of genetic variation in plant breeding (Schwarz-Sommer *et al*., 1985; Lamkey *et al*., 1991).

Marker assisted selection strategies

The success of MAS is highly dependent on the position of the marker to the gene. When the marker is within the gene, then the situation is most favorable for MAS. MAS is also very effective when the markers are in LD with the gene (i.e. the tendency of certain alleles to be inherited together). This would occur when the markers and gene are very close together. MAS has been used in cassava for the CMD2 gene (Okogbenin *et al*., 2007). MAS has also been used for cassava green mite (CGM) resistance, and can be used to select for several genes controlling one or more traits. However, the need to apply markers effectively in breeding in creating and identifying useful recombinant types, have seen the development of more robust marker-assisted selection strategies. Markers are used to identify important haplotypes and these are recombined in a recurrent selection (MARS) scheme. Markers have also been used to introgress a target gene from a donor parent into a recipient elite line in marker-assisted backcross (MABC) schemes. In MABC, markers can rapidly be used to introgress target genes and recover a full genome of a recipient genotype with few generations compared to conventional approaches.

(a) MAS

MAS is the selection of individuals with specific alleles for traits controlled by a limited number of loci (up to 6-8). The use of MAS is desirable when phenotypic screening is particularly expensive and laborious. It is very useful for pyramiding multiple resistance genes and when heritability is low. MAS involves scoring indirectly for the presence or absence of a desired phenotype of phenotypic component based on the sequences or banding patterns of molecular markers located in or near the genes controlling the phenotype (Edwards *et al*., 2004). Markers can increase screening efficiency in breeding programs by screening at the early stage for traits that are expressed late in the plant's growth cycle; by screening for traits that are extremely difficult, expensive or time consuming to score phenotypically; by distinguishing the homozygous from its heterozygous condition of many loci in a single generation without the need for progeny testing; carry out simultaneous MAS for several characters at one time (Edwards *et al*., 2004).

(b) MARS

MAS has been successfully performed for many oligogenic traits (Garland *et al*., 2000; Murai *et al*., 2001; Jia *et al*., 2002; Komori *et al*., 2003). But most agronomic traits are quantitative in nature and are the result of the joint action of several loci on chromosomes (QTL). To efficiently combine the best haplotypes for effective development of superior genotypes, MARS will best be suited to increase the frequency of favorable alleles based on a multi-parental strategy and using a "breeding by design" approach to recombine favorable alleles to build ideal haplotypes for target traits which are complex in nature. "Breeding by design" allows breeders to exploit known allelic variation to design superior genotypes by combining multiple favorable alleles (Peleman and van der Voort, 2003). MARS involves several cycles of marker-based selection and is effective in increasing the frequencies of favorable QTL or marker alleles. MARS is the identification and selection of several genomic regions (up to 20 or even more) for complex traits within a population.

Genome-wide selection (GWS)

Genome-wide selection was found most useful for complex traits controlled by many QTLs and with a low h^2. It focuses more on the genetic improvement of quantitative traits rather than understanding their genetic basis. GWS can be implemented in the same way as MARS except that all individuals would have to be genotyped with a large number of markers. Genome-wide selection (Meuwissen *et al*., 2001) focuses purely on prediction of performance based on as many loci as possible (unlimited number) and avoids QTL mapping altogether. GWS does not imply that QTL discovery should no longer be conducted, rather, the data used in GWS can be used to map QTLs (Bernardo, 2008). Therefore, GWS and QTL are not mutually exclusive. In GWS, the joint effects of all markers are fitted random effects in a linear model. Trait values are predicted from a weighted index calculated for each marker. Simulation studies have indicated that across different numbers of QTL (20, 40, and 100) and levels of h^2, responses to genome-wide selection were 18 to 43% larger than the corresponding responses to MARS (Bernardo and Yu, 2007).

Challenges and Future Trends in the Application of Molecular Markers

The utilization of molecular markers is gradually on the rise in many developing countries following increasing awareness and investments both in the private and public

sector in agricultural biotechnology for both crops and plants. Further significant increases are expected in the near future with increasing advances in their use and marked reductions in the cost of the technology.

Major differences exist between (and within) regions of the world regarding the application of molecular marker techniques in plant breeding and genetics. Although Africa has shown remarkable improvement in the uptake of this technology, its use is still relatively lower compared to other parts of the world (Sonino *et al*., 2007). The variations among regions to a large extent are a reflection in the different levels of investment in infrastructure and human resources needed to undertake research in this field. The Generation Challenge Programme (GCP) of the CGIAR has significantly invested in the application of markers in the developing world, especially in Africa and Asia, in the last decade.

Molecular markers are highly reliable selection tools, not influenced by the environment and relatively easy to score in the laboratory. Reducing the cost of molecular assays relative to phenotyping costs is one of the key considerations in the application of this technology in breeding. Cost benefit analysis is critical for the successful implementation of marker-assisted breeding. Over time, there has been considerable reduction in costs with high throughput technology rapidly making marker assays much cheaper. When markers are applied in the most efficient combination of multi-pooling and multiplexing, it could significantly lead to reductions in cost. Higher throughput systems will have lower costs per assay, as the capital and overhead costs per sample will be lower (Brennan and Martin, 2007).

To increase the volume of products obtained through the use of molecular markers, there is a need to improve the coordination between molecular marker labs and plant breeding programs, and integrating this with crop production and commercialization systems for the benefit of farmers. Applied plant breeding should be the foundation for the application of molecular markers (Sonino *et al*., 2007). The success will largely depend on the utilization of these markers under appropriate marker-assisted breeding strategies for the right traits to take best advantage of the linkage between genomic and breeding for the development of improved cultivars. The huge steps in developing appropriate MAB approaches can be very daunting, often demanding in time, efforts and expenses. For example, the development of marker assisted assays for complex traits could be extra difficult and most costly due to extensive phenotypic assays involved. However, once a good knowledge base is created to estimate appropriate parameters which efficiently determine the trait, a good experimental set up could result in the availability of marker-assisted breeding tools, which can to a major extent minimize future applications of phenotypical assays (Peleman and van der Voort, 2004).

Given the role markers have played in unlocking the genetic potential of plant genetic resources, successful gene mining of a crop's wild relatives are expected to unravel useful favorable alleles which can be pyramided to develop superior crop varieties with novel traits. The Generation Challenge Programme recently supported the establishment of an integrated breeding platform to support marker-assisted breeding programs in research centers at an efficient speed and to save costs associated with developing labs with state-of-the-art equipment, which often times is prohibitive in publicly funded research centers. The Integrated Breeding Platform seeks to reproduce and replicate the success attained in

the private sector in the public sector and to improve capacity of MAB in developing countries.

The increasing pace in genome sequencing is expected to open more vistas for molecular breeding in crops. MAB will be more effective when all gene sequences controlling plant growth and development are known. The sequence information can be used to discover genes and select for favorable alleles for target traits. Rapid advances in genomics are accumulating huge amounts of genome information, such as high density genetic and physical maps. To use this huge amount of sequence information for crop improvement, the information would have to be linked with phenotype using functional genomics, proteomics and bioinformatic tools (Young, 1999; Wilson *et al*., 2003). Given the advances in genomics, it is hoped that additional efficient molecular marker tools will be available for application in plant breeding programs to select plants with a combination of desirable alleles based on specific patterns or transport transcript expression (MacBeath and Schreiber, 2000).

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CHAPTER 10

CASSAVA PESTS IN LATIN AMERICA, AFRICA AND ASIA [1]

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ABSTRACT

The origin of cassava (Euphorbiaceae: *Manihot esculenta* Crantz) is in the Neotropical Americas, where it is estimated that domestication occurred some 7,000 to 9,000 years ago. The greatest diversity of arthropod pests attacking the crop is from this region. An estimated 200 species have been reported, many of which are specific to cassava. The pest complex varies greatly between the main cassava growing regions, indicating that careful quarantine measures could prevent pest introductions into uninfested areas. The accidental introduction of the cassava green mite (CGM: (*Mononychellus tanajoa*) and the cassava mealybug (*Phenacoccus manihoti)* from the Americas into Africa has caused devastating damage on that continent. In Asia, until recently, none of the major Neotropical cassava pests have become established, although severe infestations of red spider mites (*Tetranychus sp.*), white flies, mealybugs and white grubs are observed occasionally or tend to be localized. Agronomic characteristics such as vegetative propagation, a long growth cycle, drought tolerance, staggered planting dates and intercropping contribute to the considerable diversity of pests that feed on the crop.

The cassava pest complex can be divided into two groups: (1) those that have probably co-evolved with cassava, which is their primary or only host; and (2) generalist feeders that may attack the cassava crop sporadically or opportunistically and are often limited in geographic distribution. The first group includes the *Mononychellus* mite complex, mealybugs (*P. herreni* and *P. manihoti*) the hornworm *Erinnyis ello*, lacebugs, whiteflies, stemborers, fruitflies, shootflies, scales, thrips and gallmidges. The generalist feeders include several *Tetranychus* mite species, certain whitefly species (*Bemisia tabaci* and others), a complex of white grub species, termites, cutworms, grasshoppers, leaf-cutting ants, burrowing bugs, crickets, stemborers and others.

The most serious pests of cassava – those causing economic damage or yield loss – are generally those that have co-evolved with the crop, including mites, hornworms, whiteflies, mealybugs, lace bugs and stemborers. Generalist feeders reported causing yield losses, often on a localized basis, include *Tetranychus* (red-spider) mites, burrower bugs, white grubs, leaf-cutting ants, grasshoppers and whiteflies. Most cassava arthropod pests cause indirect plant damage given that they are foliage feeders or stemborers, reducing leaf area, leaf life or photosynthetic rate. Few cassava pests damage the roots directly. Three exceptions are white grubs, burrower bugs (*Cyrtomenus bergi*) and root mealybugs (e.g. *Pseudococcus mandio*). White grubs have been found feeding directly on cassava roots causing yield loss and severe root rot.

In general, arthropod pests are more damaging during the dry season, being less severe in areas of considerable and constant rainfall; however, there are exceptions to this rule. Hornworm and whitefly attacks often coincide with the rainy season when there is considerable new growth with young succulent leaves. Studies have also shown that burrower bugs and white grubs prefer soil with higher soil moisture content. Climate change predictions indicate that certain agricultural lands may receive less rainfall in the future. The cassava crop may have a comparative advantage in these extended seasonally drier regions; however, this could result in severer pest outbreaks that will reduce yield, or increase pesticide use. Yield losses due to mites ranged from 21-80% depending on

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length of attack, varietal susceptibility and region. A 1-, 6- and 11-month whitefly attack resulted in a 5, 42 and 79% yield loss in Colombia, respectively. Mealybug attacks in Brazil and Africa resulted in yield losses as high as 80%. Stemborer attacks in Colombia led to a 45-62% yield loss when stem breakage exceeded 35%.

A successful cassava pest management strategy minimizes or prevents chemical pesticide use and concentrates on the implementation of host plant resistance, biological control and appropriate cultural control practices. With the increased emphasis on large-scale plantations, where the cassava crop has a high commercial value, there is a tendency to apply pesticides when noticeable crop damage occurs. This should be avoided and only used as a measure of last resort. Pests that induce pesticide applications include whiteflies, mites, white grubs, hornworms, mealybugs, burrower bugs and thrips.

CIAT maintains a large germplasm bank that offers entomologists and breeders a potential pool for pest resistance genes. Moderate levels of resistance have been identified for cassava mites, but only low levels to the mealybug. High levels of resistance have been found in *M. esculenta* genotypes for whiteflies and thrips and this resistance has been incorporated in commercially released cultivars. In addition, high levels of resistance to whiteflies, mites and mealybugs have been identified in accessions (genotypes) of wild *Manihot* species (Carabalí *et al*., 2009). Interspecific crosses with *M. esculenta* have been made with the objective to introduce this resistance into commercial cultivars.

Biological control agents have been identified for many of the cassava arthropod pests but their efficacy in controlling pests in field plantations is not well documented. Classical biological control has been successful in Africa against two introduced pests from the Americas; the cassava mealybug (*P. manihoti*) and green mite *(M. tanajoa*). The mealybug *P. herreni* was successfully controlled in northeast Brazil with the introduction of parasitoids from Colombia. The use of fungal, viral and bacterial entomopathogens has shown a potential for control of several cassava pests, including whiteflies, hornworms, white grubs and burrower bugs.

Traditional farmers in many cassava-growing regions have relied on an array of cultural practices that can reduce pest populations. Intercropping has been shown to reduce populations and damage of whiteflies, hornworm, burrower bugs and stemborers. Additional cultural practices that can reduce pest populations include the selection of pest-free planting materials (scales, mites, mealybugs and stemborers), the use of varietal mixtures, destruction (burning) of plant debris (stemborers, scales), crop rotation and changed planting dates.

Quarantine measures to prevent the movement of pests, especially into Africa and Asia, are an important issue and strict regulations need to be enforced to prevent the introduction of important cassava pests into noninfested areas. Cassava pests, through the movement of vegetative planting materials, have shown the ability to disseminate great distances as evidenced by the introduction of the mite and mealybug into Africa from the Americas. There are several additional pests that could cause severe crop losses if introduced into Asia or Africa. These include several mite species, lacebugs, mealybugs, several whitefly species, stemborers, hornworm, burrower bugs and thrips. Moreover, what may be considered a secondary pest in the Neotropics could become a major pest outside its center of origin, as evidenced by the mealybug, *P. manihoti*. Vegetative materials should be introduced into a country only through tissue culture and initially grown under quarantined conditions to assure that they are free of arthropod pests and pathogens.

INTRODUCTION

The origin of cassava (Euphorbiaceae: *Manihot esculenta* Crantz) is in the Neotropical Americas, where it is estimated that domestication occurred some 7,000 to 9,000 years ago. At present, this perennial shrub is grown throughout the tropical regions of the world.

Cassava is vegetatively propagated, has a long growth cycle (8-24 months), is drought tolerant, and is often intercropped with staggered planting dates, so it is almost always present in farmers' fields. Most cassava is grown by small-scale farmers in traditional farming systems, often on marginal or fragile soils under rain-fed conditions, using few purchased inputs such as fertilizers and pesticides. As yields are low in these systems, pest control is of low priority due to the high costs and the long crop cycle, which may require various pesticide applications.

The dynamics of cassava production are changing, however, as trends in the food, feed and industrial sectors are leading to an increased demand for high-quality cassava starches. In Latin America and Asia there are indications of a shift toward larger scale production units, where cassava is grown as a plantation crop, and it is advantageous for farmers to employ a multiple planting and harvesting production system in order to meet the constant market demands of the processing industries. In this type of production system, the cassava crop will be found at several different growth stages in the same or surrounding fields. Evidence now indicates that pest problems will be compounded in these overlapping production systems. Populations of certain pests such as whiteflies, hornworms and mealybugs tend to increase when a constant food supply (e.g., young cassava foliage) is available. Given this trend, with the concomitant increase in pest populations and damage, there will be a greater tendency to apply pesticides to control pest outbreaks. There is a need to invest in cassava research in order to understand fully the role of pests and diseases in these multiple production systems, where different stages of the crop overlap, providing a constant source of nourishment.

After presenting an overview of the cassava arthropod complex and the corresponding damage to the crop, aspects of biology, behavior, and management of the most important pests are explored for the following categories: foliage feeders, stemborers/stem feeders, soil-borne pests and secondary pests.

OVERVIEW OF THE CASSAVA ARTHROPOD PEST COMPLEX AND CROP DAMAGE

Given that cassava originated in the Neotropics, the greatest diversity of arthropods reported attacking the crop is from these regions **(Table 1)**. More than 200 species have been reported, many of which are specific to cassava and have adapted in varying degrees to the array of natural biochemical defenses in the host, which include laticifers and cyanogenic compounds.

The pest complex varies greatly among the major cassava-growing areas in the Americas, Africa and Asia. The crop, whose origin is in South America (Allem, 2002), was introduced into Africa in the 1500s and into Asia in the seventeenth century. In Asia, until recently, none of the major Neotropical pests has become established. Native arthropods that have adapted to cassava have not been reported as causing serious economic damage. However, recent surveys in Thailand indicate that pest species, originally from the neotropics may be causing crop losses. The whitefly species, *Aleurodicus dispersus*, and the mealybug, *Phenacoccus manihoti* were observed in moderate to high populations in cassava fields (Bellotti, personal observations.) In Africa, the whitefly (*Bemisia tabaci*) is presently considered to be the major pest of cassava

because it is the vector of cassava mosaic disease (CMD) (Calvert and Thresh, 2002). Moreover, recent reports indicate that *B. tabaci* is also causing root yield reductions due to direct feeding on the crop. There is also the possibility of the accidental introduction of pests via planting material, which can wreck havoc. The cassava green mite (CGM: *Mononychellus tanajoa*) and the cassava mealybug (*Phenacoccus manihoti*), which were introduced from South America, have caused considerable crop losses and have been the target of massive biological control efforts in Africa (Bellotti *et al*,. 1999; Neunschwander, 2004).

Studies indicate that several arthropod species can cause considerable yield loss and that the pest complex is not geographically uniform. Two cassava mealybug species offer an example of the geographic influence on crop damage. *Phenacoccus herreni*, which has caused considerable damage in northeast Brazil, was probably introduced from northern South America (Venezuela or Colombia), where mealybug populations are controlled by natural enemies not found in Brazil. *P. manihoti*, which has caused severe crop damage in Africa, had, until recently, been reported only from Paraguay, the Mato Grosso area of Brazil and the Santa Cruz area of Bolivia. In 2005, this species was collected from the states of Bahia and Pernambuco in Northeast Brazil. The spread of *P. manihoti* into the drier, hotter regions of Brazil is probably associated with the movement of cassava planting material (i.e., stem cuttings) from southern Brazil into the Northeast.

The cassava pest complex can be divided into two groups: (i) those that have probably co-evolved with cassava, which is their primary or only host; and (ii) generalist feeders that may attack the cassava crop sporadically or opportunistically and are often limited in geographic distribution **(Table 1)**. The first group includes the *Mononychellus* mite complex, mealybugs, the hornworm *Erinnyis ello*, lacebugs, whiteflies, stemborers, fruitflies, shootflies, scales, thrips and gall midges. The generalist feeders consist mainly of a complex of white grub species, termites, cutworms, grasshoppers, leaf-cutting ants, burrower bugs, crickets, *Tetranychus* mite species and other stemborers and mealybug species (Bellotti, 2008).

The most serious pests of cassava, those causing economic damage or yield losses, are generally those that have co-evolved with the crop, including mites, hornworms, whiteflies, mealybugs, lacebugs and stemborers. Generalist feeders reported causing yield losses, often on a localized basis, include burrower bugs, white grubs, leaf-cutting ants and grasshoppers.

Most cassava arthropod pests cause indirect plant damage given that they are foliage or stem feeders, reducing leaf area, leaf life, photosynthetic rate or causing stem breakage. Those pests that can attack the crop over a prolonged period, especially during seasonally dry periods (3-6 months) can cause severe yield losses as a result of decreased photosynthesis, premature leaf drop and death of the apical meristem. Potential yield reduction by these pests can be greater than that by cyclical pests such as hornworms, leaf-cutter ants and grasshoppers, which cause sporadic defoliation; however, these highly visible pests often induce cassava producers to apply pesticides.

Table 1. Global distribution of cassava arthropod pests.

Arthropod pest	Major species	Americas	Africa	Asia
Whiteflies	*Aleurotrachelus socialis*	x		
	Aleurothrixus aepim	x		
	Aleurodicus dispersus	x	x	x
	Aleurodicus flavus	x		
	Aleuronudus sp.	x		
	Bemisia afer		x	x
	Bemisia tuberculata	x		
	Bemisia tabaci	x	x	x
	Paraleyrodes sp.	x		
	Tetraleurodes sp.	x		
	Trialeurodes vaporariorum	x		
	Trialeurodes variabilis	x		
Mealybugs	*Dysmicoccus sp.*	x		
	Ferrisia virgata(2)	x	x	x
	Maconellicoccus hirsutus(1)			x
	Phenacoccus madeirensis	x		x
	Phenacoccus manihoti	x	x	
	Phenacoccus herreni	x		
	Pseudococcus jackbeardsleyi	x		x
	Pseudococcus adonidum		x	
	Phenacoccus longispinus(1)			x
	Pseudococcus elisae(1) (8) (9)			x
Root mealybugs	*Pseudococcus mandio*	x		
	Planococcus citri (3)		x	
	Protortonia navesi	x		
	Stictococcus vayssierei		x	
Mites	*Allonychus brasiliensis*	x		
	Allonychus littoralis	x		
	Allonychus reisi	x		
	Aponychus schultzi	x		
	Atrichoproctus uncinatus	x		
	Eotetranychus falcatus		x	
	Eutetranychus africanus		x	x
	Eutetranychus banksi	x		
	Eutetranychus cratis			x
	Eutetranychus enodes		x	
	Eutetranychus orientalis		x	x
	Mononychellus bondari	x		
	Mononychellus progresivus	x	x	
	Mononychellus caribbeanae	x		
	Mononychellus chemosetosus	x		
	Mononychellus manihoti	x		
	Mononychellus mcgregori	x		x
	Mononychellus planki	x		
	Mononychellus tanajoa	x	x	
	Oligonychus peruvianus	x		

Table 1. Continued

Arthropod pest	Major species	Americas	Africa	Asia
	Oligonychus gossypii (7)	x	x	
	Oligonychus coffeae	x	x	
	Oligonychus grypus	x		
	Oligonychus biharensis		x	x
	Oligonychus mcgregori	x		
	Oligonychus thelytokus		x	x
	Oligonychus yothersi	x		
	Petrobia uncata	x		
	Tetranychus amicus		x	
	Tetranychus bellottii			x
	Tetranychus desertorum	x		
	Tetranychus escolasticae	x		
	Tetranychus gloveri	x		
	Tetranychus urticae	x	x	x
	Tetranychus bastosi	x		
	Tetranychus incestificus			x
	Tetranychus kanzawai	x		x
	Tetranychus lambi			x
	Tetranychus lombardini		x	
	Tetranychus ludeni	x		
	Tetranychus marianae			x
	Tetranychus mexicanus	x		
	Tetranychus neocaledonicus	x	x	x
	Tetranychus paschoali	x		
	Tetranychus piercei			x
	Tetranychus sayedi		x	x
	Tetranychus tumidus	x		
	Tetranychus truncatus			x
	Tetranychus yusti	x		x
Scale insects	*Aonidomytilus albus*(4)	x	x	x
	Ceroplastes sp.	x		
	Coccus viridis		x	
	Eurhizococcus sp.	x		
	Hemiberlesia sp.	x		
	Mytilaspis dispar		x	
	Monophebus sp.	x		
	Parasaissetia nigra (2)	x	x	x
	Pinnaspis minor	x		
	Saissetia coffeae		x	
	Saissetia miranda	x	x	x
	Saissetia hemisphaerica		x	
White grubs	*Anomala obsoleta*	x		
	Euchlora viridis		x	
	Euchlora pulchripes		x	
	Heteronychus plebejus		x	
	Lepidiota stigma			x
	Phyllophaga menetriesi	x		

Table 1. Continued

Arthropod pest	Major species	Americas	Africa	Asia
	Phyllophaga obsoleta	x		
	Phyllophaga sneblei	x		
	Leucopholis rorida			x
	Phyllophaga bicolor	x		
	Lepidiota stigma (9)			x
	Aserica sp.			x
	Holotrichia sp.			x
Termites	*Heterotermes tenuis*	x		
	Coptotermes paradoxis		x	
	Coptotermes spp.	x	x	x
Thrips	*Ayyaria chetophora* (1)			x
	Caliothrips masculinus	x		
	Corynothrips stenopterus	x		
	Elaphrothrips denticollis(1)			x
	Frankliniella williamsi	x	x	x
	Nesothrips lativentris(1)			x
	Retithrips syriacus			x
	Scirtothrips manihoti	x		
	Scoloptrips sp.	x		
Leafhoppers	*Empoasca bispinata*	x		
	Scaphytopius fuliginosus	x		
	Scaphytopius marginelineatus	x		
Grasshoppers	*Zonocerus elegans*		x	
	Gryllotalpa africana		x	
	Gryllotalpa sp.	x		
	Gryllus assimilis	x	x	
	Zonocerus variegatus		x	
Leaf-cutter ants	*Atta sexdens*	x		
	Atta cephalotes	x		
	Acromyrmex landolti	x		
Shootflies	*Neosilva perezi*	x		
	Lonchae chalibea	x		
	Silva pendula	x		
Fruitflies	*Anastrepha pickeli*	x		
	Anastrepha manihoti	x		
Stemborers	*Chilomima clarkei*	x		
	Chilozela bifilalis	x		
	Coelosternus alternans			
	Coleosternus granicollis	x		
	Coelosternus manihoti	x	x	
	Coelosternus notatices	x		
	Coelosternus tarpides	x		
	Coelosternus rugicollis	x		
	Eubolus sp.	x		
	Eulecriops manihoti	x		
	Lagocheirus araneiformis	x		
	Lagocheirus obsoletus	x		x

Table 1. Continued

Arthropod pest	Major species	Americas	Africa	Asia
	Lagocheirus rogersi	x		
	Lagocheirus sp.		x	x
	Dorysthenes buqueti (9)			x
	Sinoxylon brassai		x	
	Heterobosthrychus brunneus		x	
Lacebugs	*Vatiga illudens*	x		
	Vatiga. manihotae	x		
	Vatiga lunulata	x		
	Amblystira machalana	x		
Burrower bugs	*Cyrtomenus bergi*	x		
	Pangaeus piceatus	x		
	Tominotus communis	x		
Hornworm	*Erinnyis ello*	x		
	Erinnyis alope	x		
Tiger moth	*Phoenicoprocta sanguinea*	x		
	Agrotis ipsilon	x		
Army worm	*Spodoptera frugiperda*	x		
	Spodoptera eridania (6)	x		
	Spodoptera sunia	x		
Gall midge	*Iatrophobia brasiliensis*	x		
Pests of dried cassava	*Aeracerus fasciculatus*	x	x	x
	Lasioderma serricorne	x	x	x
	Rhyzopertha dominica	x	x	x
	Tribolium castaneum	x	x	x
	Sitophilus oryza	x		
	Sitophilus zeamais		x	x
	Prostephanus truncatus		x	x

(1) Capacity Building in Surveillance and Diagnosis for Leafminer, Whitefly, Thrips and Mealybug Pests in Developing APEC Economies for Improved Market Access. APEC Agricultural Technical Cooperation Working Group December 2007. www.apec.org/apec/publications/all_publications/agricultural_technical

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Few cassava pests damage cassava roots directly. Four exceptions are burrower bugs (*Cyrtomenus bergi*), white grubs (Scarabaeidae), root mealybugs (e.g., *Pseudococcus mandio*) and termites. *C. bergi* causes root punctures during feeding that can introduce fungal pathogens that reduce root yield and quality. White grubs have been found feeding directly on cassava roots causing yield loss and severe root rot. Yield losses of 17% have been reported for *P. mandio* feeding on cassava roots in southern Brazil. Termites and millipedes are reported occasionally feeding on tuberous roots; however, these may be secondary feeders, attacking already damaged and decaying roots.

In general, arthropod pests are most damaging during the dry season, being less severe in areas of considerable and consistent rainfall; however, there are exceptions to this rule. Hornworm attacks will frequently occur at the onset of the rainy season when there is considerable new growth and young leaves. Severe whitefly attacks often coincide with the rainy season when young, succulent leaves are preferred for oviposition. Studies have also shown that burrower bugs and white grubs prefer soils with higher soil moisture content.

The cassava plant is well adapted to long periods of limited water and responds to water shortage by reducing its evaporative (leaf) surface rapidly and efficiently and by partially closing the stomata, thereby increasing water-use efficiency. The crop has the potential to recover and compensate for yield losses from seasonally dry periods and pest attack due to the higher photosynthetic rate in newly formed leaves (El-Sharkawy, 1993). Younger leaves play a key role in plant carbon nutrition. Most pests prefer the younger canopy leaves; thus, dry-season feeding tends to cause the greatest yield losses in cassava.

Climate change predictions indicate that certain agricultural lands will receive less rainfall in the future. The cassava crop may have a comparative advantage in these extended seasonally drier regions; however, increased cassava production in drier regions of the Neotropics and Africa could result in severer pest outbreaks, reducing yields and/or increasing pesticide use.

Cassava yields on an average per-hectare basis, are highest in Asia (20 t/ha) and the Americas (13 t/ha) and lowest in Africa (10 t/ha). The higher yields in Asia may be due to the fact that, until recently, none of the major Neotropical cassava pests has become established.

MANAGEMENT OF CASSAVA ARTHROPOD PESTS

A. Foliage Feeders

1. Whiteflies

a. Taxonomy

Considered one of the world's most damaging agricultural pest groups, as both direct feeders and virus vectors, whiteflies attack cassava-based agroecosystems in the Americas, Africa and in Asia. Currently, they may be causing more crop damage and yield loss on cassava than any other pest attacking the crop.

There is a large species complex associated with the crop, the importance of which can vary between regions or continents. The largest complex on cassava is in the Neotropics, where 11 species are reported, including *Aleurotrachelus socialis, Trialeurodes variabilis, Aleurothrixus aepim, Bemisia tuberculata* and *Bemisia tabaci* (=*B. argentifolii*). *A. socialis* and *T. variabilis* cause considerable direct damage and yield losses in northern South America (Colombia, Venezuela and Ecuador) and in certain regions of Central America. *T. variabilis* is observed primarily in the higher altitudes (> 1000 m), while *A. socialis* is confined to lower altitudes (up to 1200 m). *A. aepim* is found in high populations causing yield losses in Northeast Brazil. *B. tuberculata* has recently been identified as causing yield losses in Southern Brazil.

Bemisia tabaci, the vector of CMD, caused by several geminiviruses, has a pantropical distribution, feeding on cassava throughout most of Africa, several countries in Asia (India and Malaysia) and more recently in the Neotropics. It has been speculated that the absence of CMD in the Americas may be related to the inability of its vector to colonize cassava effectively. Prior to the early 1990s, the *B. tabaci* biotypes found in the Americas did not feed on cassava. The B biotype of *B. tabaci*, regarded by some as a separate species (*B. argentifolii*), has been collected from cassava in several regions of the Neotropics. Although seldom observed in high populations, it is now considered that CMD poses a more serious threat to cassava production given that most traditional cultivars grown in the Neotropics are highly susceptible to the disease.

The potential of *B. tabaci* to adapt to cassava is considered a threat to cassava production in the Americas. A study was initiated to verify whether *B. tabaci* could become gradually adapted to *M. esculenta*. Trials were conducted in rearing chambers (growth rooms) measuring life cycle and population development on *B. tabaci* individuals that passed through a series of intermediate hosts, that had previously been selected and based on phylogenetic proximity to *Manihot*. The ability of *B. tabaci*, biotype B to gradually adapt to cassava (*M. esculenta*), started with individuals from a colony on a legume (*Phaseolus vulgaris*), and continued on two Euphorbiaceae, *Euphorbia pulcherrima* (poinsettia) and *Jatropha gossypiifolia*, and finally on a commercial cassava variety. The highest oviposition rate (2.66 eggs/female/2 days), the shortest development time (44.4 days) and the highest value of r_m (0.48/day) were for populations coming from *J. gossypiifolia.* 27.5% of *B. tabaci* coming from *J. gossypiifolia* survived on cassava, whereas only 3.0% survived on *E. pulcherrima* and 2.0% from *P. vulgaris* (Carabalí *et al*., 2005). The importance and potential impact of phylogenetically close plants as hosts facilitating the adaptation of *B tabaci* to cassava is evident, especially in the Neotropics where cassava is not an efficient host to *B. tabaci*. *Jatropha* is being considered as a potential source for biofuels and plans call for the planting of large plantations to this crop. This could lead to "host shifting" and cassava could become a suitable host for *B. tabaci*. This enhances the possibility of *B. tabaci* vectoring virus diseases to cassava. Studies in India (Raj *et al*., 2008) have identified a begomovirus from *Jatropha curcas* that possessed high identities and a close relationship with Indian and Sri Lankan cassava mosaic virus isolates. Precautions should be taken to insure that large *Jatropha* plantations should not be established proximate to major cassava growing regions, such as in southern Brazil.

The whitefly species *A. dispersus* is found in several cassava growing countries of Asia (India, Thailand, Laos) and may be causing yield losses. The origin of this species is in South America, possibly north of the Amazon region. Although *A. dispersus* is reported

from several countries in the Americas, it is seldom observed in high populations and no yield losses have been reported on cassava. Numerous parasitoid natural enemies have been observed in the neotropics and this may account for the low populations and lack of economic damage due to this pest. Studies need to be done in Asia, especially in Thailand, to determine the effects of *A. dispersus* on cassava yields.

b. Damage

Whiteflies can cause direct damage to cassava by feeding on the phloem of leaves, inducing leaf curling, chlorosis and defoliation. High populations, combined with prolonged feeding, result in considerable reduction in root yield. Yield losses resulting from *A. socialis* and *A. aepim* activity are common in Colombia and Brazil, respectively. With *A. socialis* feeding, there is a correlation between duration of attack and yield loss. Infestations of 1, 6 and 11 months resulted in a 5, 42 and 79% yield reduction, respectively. More recently, yield losses of 58% due to *T. variabilis* feeding have been recorded in the Andean region of northern South America (Bellotti, 2008). In several East African countries yield losses due to direct feeding by *B. tabaci* have been recorded in recent years as a result of the higher populations observed (Calvert and Thresh, 2002). In Uganda, over 50% reductions in root yield have been recorded. Observations on large cassava plantations in southern Brazil (states of Parana, Sao Paolo and Mato Grosso Sur) indicate that high *B. tuberculata* populations are reducing yields, but these losses have not been quantified.

c. Biology and behavior

Research with *A. socialis* and *A. aepim* indicates that populations of both species can occur throughout the growing cycle (one year or more) but are usually highest during the rainy season when there is considerable new growth. *A. socialis* females prefer ovipositing on the undersides of the young apical leaves, reaching a high of 244 eggs (avg 181, min. 155) per female. The individually oviposited banana-shaped eggs hatch in about ten days and pass through three feeding nymphal instars and a pupal stage (4th instar) before reaching the winged adult stage. During the third instar, the body color changes from beige to black, surrounded by a waxy white cerosine, making this species easy to distinguish from other whitefly species feeding on cassava. *A. socialis* egg-to-adult development was 32 days under growth chamber conditions (28±1°C, 70% RH). *A. socialis* may be specific to cassava, as populations have not been observed on other plant species (Holguin and Bellotti, 2004).

Aleurodicus disperses, the spiraling whitefly originates from the tropical Americas. It has been reported from many countries in Central and South America, the Caribbean, Africa (Nigeria, Benin, Congo, Togo, etc.) (Neuenschwander, 1994) and Asia (Thailand, Laos, Indonesia, India, Philippines and several others). *A. disperses*, a highly polyphagous species, has a wide host range that include many vegetables, ornamentals and fruit crops (banana, citrus, avocado, guava, soybean) as well as cassava. The immature and adult stages cause direct feeding damage that can cause premature leaf-fall. Feeding damage is accompanied by a heavy production of honeydew and a white, waxy material produced by the insect. Sooty mould develops on the honeydew and decreases photosynthetic activity. High *A. disperses* populations have been observed on cassava in Thailand; these may be causing root yield losses but this has not been documented. High *A. dispersus* populations are also reported from Benin, Africa (D'Almedia *et al*., 1998). The multiple host range of this species has probably contributed to its widespread dissemination.

The biology of this species has not been extensively studied on cassava. Adults are white, large and very visible on host plants. Adults are easily found on the under-surface of leaves where eggs are oviposited and covered in wax, in loose circular whorls. Eggs are oviposited singularly on a stalk and hatch in 4 to 10 days depending on the temperature and the host plant. The nymphal period has four instars and lasts for 12 to 14 days or longer. The 2 to 3 day pupal stage is covered with a large amount of white wax, which may act as a protective shield against natural enemies. The total life cycle ranges from 34 to 38 days (Palaniswami *et al*., 1995). Females can live up to 39 days and deposit 80 or more eggs.

A. dispersus damage symptoms include yellowish speckling of the leaves and, with severe infestations, a curling, crinkling and death of cassava leaves. Leaf damage and infestation is from bottom leaves to the top. Populations can range up to hundreds of inmature white flies per leaf. Higher whitefly populations will also result in increased sooty mold cover. In general, whiteflies require high humidity levels, especially during their developmental stages. However, high *A. dispersus* populations have been recorded during dry periods (D'Almeida *et al*., 1998).

d. Control

Integrated management of cassava whiteflies depends on having effective, low-cost, environmentally-sound technologies available for farmers. A successful whitefly control program requires continual research input to acquire the basic knowledge needed to develop the technologies and strategies for appropriate implementation. A recent survey in an important cassava-growing region of Colombia showed that 34% of the farmers surveyed applied chemical pesticides for whitefly control *versus* only 4.6% for biological products. Farmer field trials in the region revealed a 58% reduction in yield due to whitefly attack; however, 52% of the farmers surveyed employed no control measures. Pesticide applications have not provided adequate control, probably for lack of knowledge of whitefly biology, especially the immature stages (the presence of eggs and early-instar nymphs). This has resulted in inappropriate timing of applications and the misuse of chemical pesticides (Holguín and Bellotti, 2004).

Recent research and field observations on cassava whiteflies in the Neotropics indicate that control measures, especially pesticide applications, are compromised because of the whitefly's capacity for rapid population increases and its ability to develop high levels of pesticide resistance. When *A. socialis* feeds on a susceptible cassava variety, it doubles its population every 4.2 days (Holguín *et al*., 2006). Other whitefly species may follow a similar pattern. When there are overlapping crop cycles (e.g., multiple plantings) and favorable rainfall patterns, the conditions are ideal for a rapid buildup in whitefly populations as a constant food supply of young cassava leaves are available for adult feeding, high oviposition and nymphal development. Field observations indicate that once whitefly populations begin this rapid increase, they are very difficult to control, requiring repeated pesticide applications that disrupt natural biological control and that are also uneconomical for small farmers. This capacity for rapid population buildup makes it urgent to introduce efficient management practices early in the plant growth cycle, possibly during the first month of plant growth and before the economic threshold is reached. Therefore, field surveys to monitor and determine the onset of the whitefly population build-up are an important component in an IPM strategy.

Four methods of whitefly control in cassava are discussed: host plant resistance, biological, cultural and chemical.

Host plant resistance (HPR)

This form of resistance to whiteflies is rare in cultivated crops. HPR studies initiated at CIAT more than 25 years ago have systematically evaluated the accessions in the CIAT cassava germplasm bank for resistance to whiteflies, especially *A. socialis*. Of approximately 5,500 genotypes evaluated in the field in Colombia, about 75% are susceptible, with damage ratings above 3.5 (1=no damage, 6=severe damage). Emphasis is placed on those genotypes with damage ratings under 2.0 (about 8%). As there may be susceptible escapes due to insufficient selection pressure, they were reevaluated in subsequent trials. Several sources of resistance to *A. socialis* have now been identified: Genotype MEcu 72 has consistently expressed a high level of resistance, while MEcu 64, MPer 334, MPer 415 and MPer 273 express moderate-to-high levels. When feeding on resistant genotypes, *A. socialis* has less oviposition, a longer development period, smaller size and higher mortality than those feeding on susceptible genotypes. *A. socialis* nymphal instars feeding on MEcu 72 and MPer 334 suffered 72.5 and 77.5% mortality, respectively, mostly in the early instars (Bellotti and Arias, 2001). This resistance, a combination of reduced oviposition and high nymphal mortality, depresses the early buildup of whitefly populations. This allows other methods of control, such as biological control to be more effective. The early establishment of natural enemies, especially parasitoids, can be more successful in maintaining whitefly populations below economic injury levels.

A cross between MEcu 72 (female parent, whitefly resistant) and MBra 12 (male parent, high yielding, good plant type) resulted in 128 progeny, four of which were selected for whitefly resistance, yield and cooking quality. These four hybrids, along with susceptible genotypes and local farmer varieties, were evaluated at three sites in Tolima province in Colombia by CORPOICA-MADR (Colombian Corp. for Agricultural Research/Ministry of Agriculture and Rural Development) over a four-year period. The hybrid CG 489-31 was selected for high whitefly resistance, high yield and good cooking qualities. In 2003, it was officially released by MADR under the name of Nataima-31. It has attained yields of 33 t/ha, outyielding the regional farmers' variety in Tolima by 34% with no pesticide applications. Nataima-31 is now being grown commercially in several areas of Colombia and has been introduced into Ecuador and Brazil.

Given that *B. tabaci* is a pantropical species that is the vector of CMD, which causes severe cassava crop damage in Africa and India, several cassava genotypes were sent by CIAT to NRI (Natural Resources Institute-UK) to be evaluated for resistance to *B. tabaci*. Genotype MEcu 72 had the lowest rate of *B. tabaci* oviposition so it was introduced into Uganda during 2005 and will be included in a breeding program to develop whitefly-resistant varieties.

The resistance expressed in MEcu72, Natiama-31 and other genotypes should also be evaluated for resistance to *A. dispersus* in Asia where this species is being observed in high field populations.

Biological control

Numerous natural enemies are found associated with whiteflies on cassava in the Neotropics. In recent field explorations in Colombia, Ecuador, Venezuela and Brazil, a

complex of parasitoids, predators and entomopathogens were collected from several whitefly species (Bellotti *et al*., 2005). The most representative group is that of the microhymenopteran parasitoids. The richness of species in Colombia, Venezuela and Ecuador is primarily represented by the genera *Encarsia, Eretmocerus* and *Amitus*, frequently associated with *A. socialis* **(Table 2).** Gaps in knowledge about this natural enemy complex have limited the determination of their effectiveness in biological control programs. There is little knowledge regarding levels and rates of parasitism by species or specification of the host and its effect on the regulation of whitefly populations.

Eleven species of parasitoids (five genera) were collected from the cassava-growing regions of Colombia; an additional five species were collected from Ecuador and seven from Venezuela. On the Caribbean Coast of Colombia, *A. socialis* was parasitized by eight species, with the genus *Eretmocerus* comprising 70% of the parasitoids. In Magdalena province, 73% of *A. socialis* parasitism was by *Amitus macgowni*, followed by *Encarsia* sp. (26%). In the Andean region, *Eretmocerus* spp. parasitized all whitefly species, but *Encarsia pergandiella* was the predominant parasitoid of *T. variabilis*.

Greenhouse studies with *E. hispida* parasitizing *A. socialis* show that the third whitefly instar is preferred. Parasitism rates reached 75% in the third instar and 16, 45 and 43% in the first, second and fourth instars, respectively. The average parasitism rate was 45%, and peak parasitism occurred 72-96 hours after exposure (Bellotti, 2002).

Parasitoid species associated with *B. tuberculata* include *Encarsia hispida, E. pergandiella, E. sophia, E. tabacivora, Eretmocerus* sp. and others. However, there are no studies that indicate the effectiveness of these parasitoids. Research on the presence of *B. tuberculata* parasitoids in Southern Brazil, and their potential in biological control needs to be carried out.

In Northeast Brazil, the predominant whitefly species is *Aleurothrixus aepim*. Several parasitoid species have been identified, including *Encarsia porteri, E. hispida, E. aleurothrixi* and *Eretmocerus* sp. However, there is little information available on the effectiveness of these natural enemies (Farias and Bellotti, 2006).

Several parasitoid species have been recorded parasitizing *A. disperses*; these include *Aleurotonus vittatus, Encarsia* sp., *E. haitiensis, E. guadeloupae, Eretmocerus* sp. and *Euderomphale* sp. *E. haitiensis* and *E. guadeloupae* (both species probably from the Caribbean region) have been shown to be effective in reducing *A. dispersus* populations in Benin, Africa (D'Almeida *et al*., 1998). It is estimated that these two species were accidentally introduced, along with *A. dispersus*, into Benin during the early 1990s. *A. dispersus* populations in Asian countries need to be surveyed in order to determine if these two parasitoids, or other parasitoids, are present and their effectiveness evaluated. *E. haitiensis* and *E. guadeloupae* are both reported as parasites of *A. disperses* in the Philippines and Malaysia **(Table 2)**.

Table 2. Natural enemies (parasitoids, predators and entomopathogens) of white flies feeding on cassava.

Principal Species	*Parasitoids*	*Predators*	*Entomopathogens*
Aleurotrachelus sociales	*Amitus macgowni* *E. americana* *E. bellotti* *E. cubensis* *Encarsia hispida* *E. luteola* *E. sophia* *Encarsia sp. nr. variegata* *Encarsia sp.* *E. tabacivora* *Euderomphale sp.* *Eretmocerus spp.* *Metaphycus sp.* *Signiphora aleyrodis*	*Delphastus sp* *D. quinculus* *D. pusillus* *Chrysopa sp. nr. cincta* *Condylostylus sp.*	*Beauveria bassiana* *lecanicillium lecani* *Aschersonia aleyrodes*
Aleurothrixus aepim	*Encarsia porteri* *E. aleurothrixi* *E. hispida* *Eretmocerus sp.*		*Cladosporium sp.*
Aleurodicus dispersus	*Aleurotonus vittatus* *E. haitiensis* *Encarsia sp.* *Eretmocerus sp.* *Euderomphale sp.*		
Aleuroglandulus similis	*Encarsia guadeloupae* *Encarsia desantisi*	*Nephaspis namolica*	
Aonidomytilus albus	*Aspidoiphagus citrinus* *Signiphora sp.*	*Chilocorus distigma*	*Septobasidium sp.*
Bemisia tuberculata	*E. hispida* *E. pergandiella* *E. sophia* *Encarsia sp.prob. variegata* *E. tabacivora* *Eretmocerus sp.* *Euderomphale sp.* *Metaphycus sp.*	*Condylostylus sp.*	
Bemisia tabaci	*Encarsia sophia* *E. lutea* *E. Formosa* *E. mineoi* *Encarsia sp.* *Eretmocerus mundus*	*Delphastus pusillus* *Condylostylus sp.*	

Table 2. Continued

Principal Species	*Parasitoids*	*Predators*	*Entomopathogens*
Trialeurodes variabilis	*E. bellotti* *E. hispida* *E. luteola* *E. nigricephala* *E. pergandiella* *Encarsia sp.* *E. sophia* *E. strenua* *E. tabacivora* *Eretmocerus spp.*	*Chrysopa sp. nr. cincta* *Condylostylus sp.*	*Aschersonia aleyrodes* *Beauveria bassiana* *Lecanicillium lecani*
Trialeurodes vaporariorum	*Encarsia tabacivora*		

More than 20 species of entomopathogens have been reported infecting whiteflies on cassava, including *Aschersonia* sp., *Lecanicillium* (*Verticillium*) *lecani, Beauveria bassiana* and *Paecilomyces fumosoroseus*. However, there has to be a careful selection of the species, as well as the identification of native isolates of entomopathogenic fungi. Greenhouse experiments at CIAT with an isolate of *L. lecani* resulted in 58-72% *A. socialis* nymphal mortality and 82% egg mortality. The *L. lecani* isolate has been formulated into a commercial biopesticide BioCanii®. The commercial biopesticide Mycotrol®, (isolate of *B. bassiana*, a product of Laverlam S.A.), gave very effective control (>90% mortality of the egg and first two nymphal instar stages) of *A. socialis* in greenhouse experiments at CIAT. Mycotrol®, was also effective against *B. tabaci* and *T. variabilis*, but needs to be evaluated in field trials.

The employment of biopesticides (e.g. fungal entomopathogens) for whitefly control appears to be most effective if applied when whitefly populations are low and in the egg and nymphal stages. Field experiments indicate that biopesticides, if applied when whitefly populations are high, do not deter the continual increase of the population. The combination of a resistant variety with applications of a biopesticide (if needed) would probably be very effective in maintaining whitefly populations below economic injury levels.

The most frequently observed predators feeding on cassava whiteflies are crysopids (Neuroptera: Crysopidae). These generalists feed on the eggs and immature stages of numerous arthropods. *Chrysoperla carnea* is frequently collected feeding on *A. socialis* in cassava fields. In lab studies at CIAT, *A. socialis* egg and nymphal consumption by *C. carnea* were measured by recording the time required for 50% consumption of the prey state being offered. *C. carnea* adults required 80 hours to consume 50% of the nymphal instars and pupae and 77 hours to consume 50% of the eggs.

Cultural control

In traditional cropping systems cassava is often intercropped, a practice that has been shown to reduce populations of certain pests. Intercropping cassava with cowpeas reduced egg populations of *A. socialis* and *T. variabilis* by 70%, compared to those in

monoculture. Yield losses in cassava/maize, cassava monoculture and mixed cultivar systems were ca. 60% versus only 12% in cassava/cowpea intercrops (Gold *et al*., 1989).

When cassava is grown in overlapping cycles or multiple plantings, it is difficult to 'break' the whitefly development cycle so rapid population buildups occur. Upon emerging from the pupal stage, adults migrate to feed and oviposit on recently germinated young plants in adjacent fields. A successful tactic for countering this situation is to implement a 'closed season', defined as an interdiction or prohibition when cassava cannot be present in the field. Field observations at CIAT have shown that a 1- to 2-month period with no cassava in the field decreases whitefly populations dramatically over a four-year period. The success of this ban is enhanced by the fact that *A. socialis* does not appear to have efficient alternate hosts so their populations 'crash' when adults cannot find an alternate host species to sustain or increase populations. Nevertheless, the economic practicality of this strategy for producers is debatable. In many regions, a constant supply of cassava roots is economically desirable for meeting the demands of local fresh and processing markets. This same tactic may not be as effective for a species such as *A. dispersus* and *B. tabaci*, both of which have numerous alternate hosts on which their populations can be sustained and multiplied.

Chemical control

Several products with new or novel active ingredients have been evaluated for controlling *A. socialis* and *T. variabilis*. Foliar applications of Thaimethoxam and Imidacloprid were most efficient in reducing whitefly populations. Best control was obtained when applied as a drench at a high dose (0.8 and 0.6 l/ha) on young plants (Holguín and Bellotti, 2004). The treatment of cassava planting material (stem cuttings) with a 7-min. emersion in a solution of Thaimethoxam (Actara®) (1 g/l H_2O) is also giving promising results. More than two pesticide applications during the crop cycle should be avoided. Pesticide applications should be made when whitefly populations are still low. Pesticides are used as a deterrent to the rapid build up of whitefly populations. Field experiments have shown that high whitefly populations are difficult to control with chemical pesticides, even when applied at high doses. Field experiments have also shown that pesticides need not be applied after six months of crop growth as yield loss due to whitefly attack will not occur. A cost-benefit analysis indicates that chemical pesticide applications for whitefly control in cassava are generally uneconomic, and only slightly economic when the cassava root price is high. Research is under way to evaluate the feasibility of substituting entomopathogens as biopesticides to replace chemical pesticide applications (Holguin and Bellotti, 2004)..

e. Recommendations for whitefly management in cassava

Resistant varieties: Nataima 31

Stake treatment: Immersion of cassava cuttings in a solution of Thaimethoxam (Actara), 1,0 gr/l water, for 7-10 minutes

Foliar application:

1. Monitor the crop every five days after the first leaves appear; evaluate the whitefly population (egg, nymphs, adults)
2. When adult populations reach 50 per plant (and 200 nymphs) apply Imidacloprid-B-Cyflutrina (Probado Combi®) and Buprofezin (Oportune®) at 4,0 and 1.0 cc/l water, respectively. The application is applied to the undersurface of leaves

3. Continue to monitor for six months:
   - >200 adults and nymphs, apply Thaimethoxam
   - 10-20 adults and few and no nymphs, apply Etoferprox (Trebon®), 5 cc/l water
   - Low population of adults, eggs and nymphs, apply fungal entomopathogens, *Lecanicillium lecani* or *Beauveria bassiana*.

2. Cassava mealybugs

a. Taxonomy

Approximately 15 species of mealybugs are reported attacking the cassava crop **(Table 1)**. Two species, *Phenacoccus herreri* and *P. manihoti*, are economically important in the Americas where they have caused yield reductions in cassava fields (Bellotti, 2008). *P. manihoti* was introduced inadvertently into Africa in the early 1970s, where it spread rapidly across the cassava-growing regions of that continent, causing considerable yield loss. Both species are of neotropical (the Americas) origin and both species have been the object of successful biological control programs (Neuenschwander, 2004; Bento *et al*., 2000). In the Americas, *P. manihoti* was first found in Paraguay in 1980 and was later collected from certain areas of Bolivia and Mato Grosso do Sul state in Brazil, causing no economic damage. More recently, *P. manihoti* has also been collected from the Brazilian states of Parana, Sao Paulo, Bahia and Pernambuco where it appears to be causing losses of cassava root yields. *P. manihoti* was probably introduced into the northeastern states of Bahia and Pernambuco through the introduction of cassava stems for planting material from Southern Brazil.

The origin of *P. herreni* is probably northern South America, where it was found in cassava-growing regions of Colombia and Venezuela. It was first reported during the mid-1970s in Northeast Brazil, where high populations caused considerable yield losses. Surveys in the region found few parasitoid natural enemies, suggesting that *P. herreni* is an exotic pest, probably coming from northern South America where parasitoids are frequently observed (Bento *et al*., 2000).

During the past year (2008/09) the mealybug species *Dysmicoccus sp*. has been observed attacking the roots of young, recently germinated, cassava plants in Southern Brazil. Damage symptoms include the wilting of young leaves, often resulting in plant mortality (Fadel, peronal communication).

Recent mealybug collections from cassava fields in Asia, especially in Thailand, indicate that there may be several species involved. There is an urgent need to do continual collections from different regions and countries to clarify this situation. The striped mealybug, *Ferrisia virgata*, has been reported feeding on cassava in Asia for many years, but was never observed in very high populations. Recent observations indicate that populations have increased dramatically and are now causing yield losses (Bellotti, pers. obs.). A second mealybug species recently collected from cassava appears to be *Pseudococcus jackbeardsleyi* (identification pending). A third species could be *Pseudococcus elisae*; this species is closely related to *P. jackbeardsleyi* and was reported on cassava in Thailand (and other Asian countries) in 1998. A fourth species, *Phenacoccus solenopsis*, is reported as a recent introduction into Asia. A fifth species has recently been identified by taxonomists at the British Museum as *Phenacoccus manihoti*, and it has been observed in high populations causing yield losses estimated as high as 25% in Thailand. Recent reports indicate that *P. manihoti* is also present and causing damage to cassava in

Cambodia. All five of these species are native of the Americas and are reported on several other hosts besides cassava. *Phenacoccus hirsutus* is reported to be feeding on cassava in the Philippines.

b. Damage

P. manihoti and *P. herreni* cause similar damage: adult and nymph feeding causes leaf yellowing, curling and cabbage-like malformation of the apical growing points. High populations lead to leaf necrosis, defoliation, stem distortion and shoot death (Bellotti *et al*., 1999). Reductions in photosynthetic rate, transpiration and mesophyll efficiency – together with moderate increases in water-pressure deficit, internal CO_2 and leaf temperature – were found in infested plants (Polanía *et al*., 1999). Yield losses in experimental fields at CIAT ranged from 68-88%, depending on cultivar susceptibility. Farmers in NE Brazil estimated their losses to be over 80%, and cassava production decreased in the region during the 1980s. In Africa yield losses due to *P. manihoti* feeding and damage were around 80%.

In general, mealybugs can cause two types of damage to cassava; a mechanical or direct damage caused by their sucking feeding habits, and an indirect damage produced by the build-up of sooty mold on the leaf surface due to mealybug excrement. This fungus build-up reduces leaf photosynthesis. *Ferrisia virgata*, the striped mealybug, can often be found in high populations feeding on the undersurface of leaves and in clusters along the stems and branches of the cassava plant. When high populations occur, considerable sooty mold can be observed. *F.virgata* causes leaf yellowing and eventually defoliation, usually beginning with the basal leaves. The mealybug infestation can spread rapidly eventually covering most leaves, stems and shoots. When high populations occur on young plants, growth is slowed, resulting in stunted and dwarfed plants. Stems will have shortened internodes, shoots and leaves are deformed and wilting occurs, eventually leading to leaf and shoot desiccation and defoliation.

High *F. virgata* populations have occurred in certain regions of Thailand in recent years, specially in areas where the rainy season was delayed, prolonging the dry season. It was estimated that yields were reduced by 20 to 80% in fields where high populations occurred.

c. Biology and behavior

In general, mealybugs are all very much alike in their life history and biology. Mealybugs may be placed into two groups: The short-tailed mealybugs and the long-tailed mealybugs. The short-tailed mealybugs reproduce by laying eggs, often in an ovisac. The filaments that surround the body are of about equal length and none more than one-fourth the length of the body. The long-tailed mealybugs generally do not form an egg sac, giving birth to live nymphs. It derives its name from the four filaments near the tip of the abdomen, which may be as long as the body. *P. herreni* and *P. maniloti* are short-tailed species, while *F. virgata* is a long-tailed species.

Mealybugs are oval, flattened, soft-bodied insects, distinctly segmented but without a clear definition between the head, thorax and abdomen. They are covered with a white, powdery, or mealy wax and feed by inserting their slender mouth parts into the plant tissues and sucking cell contents. Mealybugs pass through four stages, egg, nymph, pupae and adults. Nymphs and adults will move about to some extent over the plant, and although

sluggish, do not remain fixed. The female nymphs change little in their appearance, except to increase in size, and they are not winged in the adult stage. The males will enter a cocoon-like pupal stage and emerge as tiny active, two-winged insects. Upon emerging from the cocoon, the males actively fly about and mate with the females. Adult males do not feed whereas adult females can continue to feed.

Both *Phenacoccus herreni* and *P. manihoti* are morphologically similar and originally thought to be only one species. *P manihoti* is parthenogenic, whereas males are required for reproduction of *P. herreni* (Bellotti, 2008). The females deposit ovisacs containing hundreds of eggs on the undersides of leaves and around apical and lateral buds. Eggs hatch in 6-8 days, and there are four nymphal instars; the first instars are highly mobile and will spread over the plant or between plants. The fourth instar is the adult stage for females, while males have four nymphal instars plus the adult stage. The third and fourth instars occur in a cocoon, from which the winged male adults emerge, living only 2-4 days. The life cycle of the female is 49.5 days; that of the male, 29.5. The optimal temperature for female development is 25-30ºC (Herrera *et al*., 1989). Populations of both species peak during the dry season (Calatayud *et al*., 2002). The onset of rains reduces pest populations and plant damage, permitting some crop recovery.

Ferrisia virgata females are described as flat bodied, 1.8 x 3.0 mm with a tail about 1.6 mm long. The winged male body is about 0.5 x 1.3 mm. The life cycle has been recorded as 35 to 92 days and females can oviposit on average 364 eggs. The adult female is covered with a powdery white wax and has a pair of purplish dorsal stripes along the back. Long, glossy white wax threads extend from the body and there are two long waxy tails. Although mobile, they generally do not move very far and large clusters of the mealybug can appear (Schreiner, 2000). *F. virgata* can attack a wide host range that includes sweetpotato, coffee, cacao, citrus, guava, tomatoes and eggplants, as well as cassava (Schreiner, 2000).

Pseudococcus jackbeardsleyi has not been reported as a serious pest and there is little information available on this species. It has both a wide distribution and host range. Commonly known as the Jack Beardsley mealybug, it is reported on a diverse range of fruits, vegetables and ornamentals, as well as cassava. Hosts include pineapple, cherimoya, celery, cabbage, pigeon pea, bell peppers, star apple, grapefruit, melon, banana, beans and numerous others (from 88 genera and 38 plant families). It has been reported from nearly all countries of the tropical Americas and several countries in Africa and Asia.

Mealybug dissemination between regions, countries or continents is probably through infested stem cuttings. The introduction of *P. manihoti* into NE Brazil from Southern Brazil can probably be traced to the movement of cassava varieties between these two regions.

Immature mealybugs can be found around the lateral buds on cassava stems, and subsequently on the stem cuttings used as planting material. If infested stem cutting are transported from one region to another, this can result in infesting an area where the mealybug was not previously established. The planting of infested stem cuttings also results in mealybug infestations from one crop cycle to the next.

d. Management

Mealybugs can be effectively controlled through the proper employment of biological control agents, especially parasitoids. The use of chemical pesticides for mealybug control can be both difficult and costly. The objective is to prevent mealybug populations from reaching economic damage (yield loss) levels. Therefore, any mealybug management action need to be employed when pest populations are still low. Unless there is constant monitoring of pest populations in the field, it is often difficult to detect the initial build up of mealybug populations. This is especially true on larger cassava plantations, where a monitoring scheme is often not implemented. The establishment and presence of key or effective natural enemies, especially parasitoids, can prevent or retard the initial build up of the mealybug populations. The employment of a chemical pesticide can be disruptive to the natural biological control that exists, or is introduced into a cassava field. Most natural enemies, especially parasitoids, are very sensitive to pesticides, even when they are applied at low doses. In contrast to biological control methods, the use of chemical pesticides does not require as much knowledge of the ecological origins of the pest. Effective biological control requires considerable knowledge and information on the origin, biology, ecology, behavior and taxonomy of both the pest and their natural enemies (Van Driesche and Bellows, 1996). Cassava mealybug management is a well-documented example of classical biological control, in both Africa and the Americas. In Africa, *P. manihoti* is being controlled successfully after introducing the parasitoid *Anagyrus lopezi* from the Neotropics. After several years of exploration in the Neotropics by scientists from IIBC, IITA and CIAT, the target species *P. manihoti* was finally located by a CIAT scientist (A.C. Bellotti) in Paraguay in 1980. IIBC collected natural enemies of *P. manihoti* that were sent via quarantine in London to IITA in Benin for multiplication and release in Africa. The encyrtid parasitoid *A. lopezi* and the coccinellid predators *Hyperaspis notata*, *Hyperaspis raynevali*, and *Diomus* sp. became established in Africa. The parasitoid is credited with being the principal agent reducing the mealybug populations. *A. lopezi* became established in all ecological zones occupied by *P. manihoti* and is now found in 27 countries, covering an area of 2.7 million km^2. Cassava losses have been reduced by 90-95% with an estimated savings of US$ 7.971 to 20.226 billion (Neuenschwander, 2004).

Surveys in Colombia and Venezuela identified numerous parasitoids, predators and entomopathogens associated with *P. herreni* **(Table 3)**. Several parasitoids show a specificity or preference for *P. herreni*: *Acerophagus coccois, Anagyrus diversicornis, Anagyrus putonophilus, Anagyrus isolitus, Anagyrus elegeri* and *Aenasius vexans*. Based on numerous field and lab studies, three encyrtid parasitoids (*A. diversicornis, A. coccois* and *A. vexans*) were identified as effective in reducing *P. herreni* infestations (Van Driesche *et al*., 1988). Comparative life-cycle studies show that they completed two cycles for each cycle of *P. herreni.* This is a favorable ratio for biological control. *A. diversicornis* prefers third instar nymphs, whereas the smaller *A. coccois* parasitizes male cocoons, adult females, and second instar nymphs. *A. vexans* prefers second and third instar nymphs. Field studies with natural populations of *A. diversicornis* and *A. coccois* estimated *P. herreni* mortality at 55% for their combined action.

Table 3. Natural enemies (parasitoids, predators and entomopathogens) of mealybugs feeding on cassava.

Principal Species	*Parasitoids*	*Predators*	*Entomopathogens*
Phenacoccus manihoti	*Anagyrus lopezi* *Acerophagus sp.*	*Cleothera onerata,* *Hyperaspis sp.* *Nephus sp.* *Chrysopa sp.* *Sympherobius sp.,* *Typhlodromalus aripo*	
Phenacoccus herreni	*Acerophagus coccois* *Apoanagyrus diversicornis* *Aenasius vexans,* *Anagyrus insolitus* *A. thyridopterygis* *A. pseudococci* *Anagyrus. sp. nr. greeni* *Aenasius sp.* nr. *putonophylus* *Prochiloneurus dactylopii* *Chartocerus sp.* *Hexacnemus sp.* *Eusemion sp.*	Ocyptamus *sp.* Sympherobius *sp.* *Hyperaspis sp.* *Nephus sp.* *Cleothera onerata* *C. notata* *Diomus sp.* *Coccidophylus sp* *Scymnus sp.* *Olla sp.* *Curinus colombianus* *Cycloneda sanguinea,* *Hippodamia convergens* *Azya sp.* *Chrysopa sp.* *Kalodiplosis coccidarum,* *Zelus sp.*	*Cladosporium sp.* *Neozygites fumosa*
Phenacoccus madeirensis	*Anagyrus sp.* *Apoanagyrus sp.* *Aenasius masii* *Acerophagus coccois* *Hexacnemus sp.* *Eusemion sp.* *Haltichella sp.* *Prospaltella sp.* *Signiphora sp.*	*Azya sp.* *Curinus colombianus* *Cleothera onerata* *Chrysopa sp.* *Coccidophylus sp.* *Emesaya sp.* *Hippodamia convergens* *Kalodiplosis coccidarum* *Nephus sp.* *Ocyptamus sp.* *Olla sp.* *Pentillia sp.* *Scymnus sp.* *Sympherobius sp.* *Zelus sp.*	*Cladosporium sp.*

Table 3. Continued

Principal Species	*Parasitoids*	*Predators*	*Entomopathogens*
Ferrisia virgata	*Aenasius advena*	*Alloagrapta javana*	*Empusa fresenii*
	Anagyrus brevicornis	*Alloagrapta obliqua*	
	Anagyrus qadrii	*Azya luteipes*	
	Anaysis alcocki	*Cheilomenes*	
	Anusioptera aureocincta	*sexmaculata*	
	Blepyrus insularis	*Chrysopa orestes*	
	Coelinius	*Exochomus flaviventris*	
	Gyranusoidea citrina	*Hyperaspis senegalensis*	
	Myiopharus doryphorae	*hottentotta*	
	Patiyana coccorum	*Mallada boninensis*	
	Pseudaphycus debachi	*Nephus regularis*	
		Scymnus castaneus	
		Scymnus coccivora	

Through the combined efforts of CIAT and CNPMF/EMBRAPA, these three parasitoids were exported from CIAT to EMBRAPA, Brazil, where they were mass reared and released into *P. herreni*-infested cassava fields, primarily in the northeastern states of Bahia and Pernambuco from 1994 to 1996. More than 35,000 parasitoids were released, and all three species became established. Studies prior to release had determined that none of these species existed in this region. In Bahia, *A. diversicornis* dispersed 120 km in six months after release and 304 km in 21 months. *A. coccois* was recovered in high numbers nine months later, 180 km from its release site. *A. vexans* was consistently recaptured at its release site in Pernambuco, having dispersed only 40 km in five months (Bento *et al*., 2000).

Personal observations in recent years indicate that *P. herreni* populations have decreased considerably as cassava farmers in the region have not reported severe outbreaks, and cassava cultivation has returned to areas where it had been previously abandoned due to *P. herreni* damage. However, the recent introduction of *P. manihoti* into the region has resulted in reports of severe mealybug damage in Bahia, causing alarm among cassava producers. An effort by local institutions and researchers is needed to determine if key *P. manihoti* parasitoids are present or need to be introduced into the region. *P. manihoti* was probably introduced into Northeast Brazil via infested cassava stems, transported from Southern Brazil and used as planting material.

Southern Brazil (the States of Parana, Sao Paulo and Mato Grosso) has recently experienced considerable damage to the cassava crop due to increased populations of the mealybug *P. manihoti*. This species has been in the region for many years and Southern Brazil, Paraguay, Northern Argentina appears to be the center of origin of this species. However, it was not previously observed in high populations, causing economic damage to the cassava crop. There are probably several factors that have contributed to the increase in *P. manihoti* populations in this region. Cassava monoculture production on large plantations has provided ample vegetative material for mealybugs to feed on and rapidly increase in population.

Changes in the climate, with a warmer "winter" period (June to August), has altered crop management practices enabling a staggered or more frequent plantings of

cassava. This results in multiple planting systems with the cassava crop at several different growth stages in the same or surrounding plantations. Observations indicate that this multiple planting system is favorable for increased mealybug populations, leading to yield losses. In addition, the increased use of chemical pesticides to control whiteflies and lacebugs, two important pests in the region, has probably reduced the effectiveness of the natural biological control agents, especially the parasitoid *A. lopezi*.

As stated earlier, mealybug populations in Asia, especially in Thailand have also increased in recent years. Factors such as warmer temperatures, a longer dry season, or the use of pesticides may have contributed to these increases in mealybug populations and damage. The two major species collected from Thailand have been identified as *F. virgata* and *P. jackbeardsleyi*, both of neotropical (the Americas) origin. Several parasitoid species of *F. virgata* have been reported **(Table 3)** but there is little data on field efficacy.

A third mealybug species, *Phenacoccus manihoti*, has now been identified in Thailand where it is causing severe crop damage. The genus *Phenacoccus* is of neotropical origin. *P. manihoti* is specific to cassava and does not appear to have additional major hosts. This species can cause considerable cassava yield losses, especially in areas of a long dry season (3 to 6 months). A classical biological control program that includes the introduction of key natural enemies from the Americas is recommended.

A concerted research effort and surveys need to be undertaken in the neotropics to identify, evaluate and research the most important natural enemies, especially parasitoids of *F. virgata, P. jackbeardsleyi and P. madeirensis.*

e. Recommendations for control of cassava mealybug.

Strategies for prevention:

Knowledge of biology (life cycle) ecology and behavior of pest species.

1. Selection and treatment of planting material (Thaimethoxam) in areas of high pest pressure.
2. Minimize movements from infested to non-infested fields. Enforce quarantine regulations.
3. Avoid use of chemical pesticides (conserve mealybug natural enemies-parasitoids).

Control Strategies

4. Constant monitoring of plantations (every 2-4 weeks).
5. Detect focal point of infestation (hot spots).
6. Focal point: Remove infested growing areas of plant (apical buds) and destroy (burn).
7. Application of a systemic pesticide in area of infestation and surrounding area.
8. Release and establishment of natural enemies (depending on mealybug species).
9. Avoid movement of planting material (stem cuttings) from one region to another.

3. Cassava mites

a. Taxonomy

More than 40 species of mites have been reported to feed on cassava in the Americas, Africa and Asia. The most important are *Mononychellus tanajoa* (syn=*M. progresivus*), *Mononychellus caribbeanae*, *Tetranychus cinnabarinus* and *Tetranychus urticae* (also reported as *T. bimaculatus* and *T. telarius*) **(Table 1)**. Cassava is the major host for the *Mononychellus* spp., while the *Tetranychus* spp. have a wide host range. Mite species reported from Asian cassava fields include *Tetranychus urticae*, *T. tumidus*, *T. kanzawai*, *T. bellotti*, *T. neocalidonicus*, *T. truncates*, *Olygonychus biharensis* and *Eutetranychus orientalis*. *M. mcgregori* has recently been identified from cassava fields in Vietnam (Bellotti, pers. obs. 2009).

M. tanajoa, the Cassava Green Mite (CGM), is the most important species, causing crop losses in the Americas and Africa. It is native to the Neotropics, first being reported from northeast Brazil in 1938. It is presently found in most cassava-growing regions in the Americas, especially in seasonally dry regions of the lowland tropics in Brazil, Colombia and Venezuela (Bellotti *et al*., 1999). *M. tanajoa* is reported in most cassava growing countries of Africa where it can cause severe crop damage (Herren and Neuenschwander, 1991). *M. tanajoa* has not been reported from any of the Asian cassava growing countries. However, climatic conditions in several Asian countries are favorable for *M. tanajoa* invasion and high population buildups. Every precaution should be taken to prevent the introduction of *M. tanajoa* into Asian countries and strict quarantine regulations should be observed.

b. Damage

In experimental fields in Colombia, *M. tanajoa* attacks of 3, 4 and 6 months resulted in yield losses of 21, 25 and 53%, respectively. Under high mite populations on the Colombian Atlantic Coast, yields were reduced by 15% in resistant cultivars compared with an average 67% loss in susceptible cultivars (Bellotti, 2008). In Africa *M. tanajoa* was first reported from Uganda in 1971; and within 15 years it had spread across most of the cassava-growing belt, occurring in 27 countries and causing estimated root losses of 13-80%. CGM has been the objective of a major biological control effort since the early 1980s (Yaninek *et al*., 1993).

Yield losses due to the Red Spider Mites, *Tetranychus* sp., feeding on cassava have been reported from the Philippines, Indonesia and India. Yield losses ranging from 18 to 47% have been recorded in field trials. The mite species involved include *T. kanzawai* (Philippines), *E. orientalis*, *T. neocalidonicus* and *O. biharensis* (India) and *Tetranychus* sp. (Indonesia).

Mite attacks will reduce the quality and quantity of planting material (cuttings) as well as decrease the root yields.

c. Biology and behavior

Mites, especially the CGM, are dry-season pests that can cause yield losses where there is a seasonally dry period of at least three months. At the onset of the rainy season, mite populations decrease and cassava plants produce new foliage. If the rains do not persist, CGM populations will again increase, causing defoliation and severer yield losses. This pattern has been observed in the semi-arid cassava-growing regions of Northeast Brazil. CGM populations prefer to feed on the undersides of young emerging leaves, by penetration of the stylet into leaf tissue, sucking cell content. Leaves develop a mottled

whitish-to-yellow appearance and may become deformed or reduced in size. Heavy infestations will cause defoliations, beginning at the top of the plant, often killing apical and lateral buds and shoots.

The adult is green in color with an average body length of about 350 mμ. Females oviposit on the leaf undersurface; eggs hatch in 3-4 days (30°C and 70±5% RH). At 15, 20, 25 and 30°C, the egg-to-adult stage is 41.4, 19.5, 10.3 and 7.8 days, respectively. These data indicate that CGM populations can increase rapidly in warm regions of the lowland tropics. At 30°C, each female oviposits 90-120 eggs; during the initial population buildup, mostly females are produced, adding to the rapid population increase (Bellotti, 2008).

Mites of the genus *Tetranychus* tend to first attack mature leaves at the basal part of the plant, then move to the upper leaves. First symptoms generally occur at the base of the leaf and along the midrib. *Tetranychus* mite colonies feed on the lower leaf surface, but in the case of heavy infestations, they attack both leaf surfaces, often causing considerable webbing. Initial spotting becomes reddish or rust-colored as the infestation increases; defoliation occurs from bottom to top leaves and, if dry conditions persist, plants may die (Bernardo and Esquevia, 1981).

Tetranychidae mites pass through five development stages: egg, larvae, protonymph, deutonymph and adult. Mites have a rapid development rate and their reproductive capacity is influenced by relative humidity, temperature, plant variety, cellular nutrition and the presence of effective natural enemies.

Tetranychus urticae females oviposit on the leaf undersurface; eggs hatch in 3-4 days; and the egg to adult stage (25 to 28ºC, RH 60-70%) is 7 to 11 days. Higher temperatures favor a higher net reproductive rate, a shorter generational time, a higher intrinsic rate of population growth and a shorter doubling time of the population.

d. Management

Pesticide applications for controlling mites on a long-cycle crop such as cassava are not a feasible or economic option for low-income farmers. Moreover, even low doses of pesticides have adverse effects on natural enemies. Cultural control methods have not been explored, and there is little mention of their use in the literature. Research into the control of *M. tanajoa* and *T. urticae* has followed two main thrusts: host plant resistance and biological control. It is expected that these two complementary strategies can reduce mite populations below economic injury levels.

Host plant resistance (HPR)

It is hypothesized that in the presence of efficacious natural enemies, only low-to-moderate levels of HPR are needed to reduce CGM populations below economic injury levels. A level of resistance that would hinder, delay or suppress the initial buildup of CGM populations could provide sufficient opportunity for establishing effective natural enemy populations that would prevent an eruption of the CGM population. Therefore, an important objective of an HPR strategy is to develop cultivars that are not highly susceptible to the CGM and that hopefully contain low-to-moderate levels of resistance. Immunity or even high levels of resistance do not appear to be available in *M. esculenta* germplasm.

A considerable effort has been made to identify CGM resistance in cultivated cassava. CIAT, IITA and several national research programs in the Americas and Africa have screened cassava germplasm for CGM resistance. Of the more than 5,000 landrace cultivars in the CIAT cassava germplasm bank, only 6% (300 cvs.) were identified as having low-to-moderate levels of resistance. A select number of cultivars with moderate levels of resistance have been released to farmers after a considerable effort by plant breeders and entomologists. Two hybrids (ICA Costeña and Nataima 31), both with low levels of mite resistance, are being grown by cassava farmers in Colombia (Bellotti, 2008).

Most mite-resistance field evaluations by CIAT have been carried out at the Colombian Atlantic Coast in the lowland tropics with a prolonged dry season (4-6 months) and high mite populations. In Brazil, CGM evaluations were conducted by CNPMF/EMBRAPA, primarily in the semi-arid regions of the Northeast. Of the 300 cultivars identified by CIAT as promising for CGM resistance (over several years and 2-7 field cycles), 72 have consistently had damage ratings below 3.0. Low-to-moderate levels of resistance are indicated by 0-3.5 (0-6 damage scale).

Mite resistance-mechanism studies indicate strong antizenosis (preference vs non-preference) for oviposition, as well as moderate antibiosis. In lab studies, *M. tanajoa* displayed a strong ovipositional preference for susceptible varieties. When paired with the moderately resistant cvs. MEcu 72, MPer 611 and MEcu 64 in free-choice tests, 95, 91 and 88%, respectively, of the eggs were oviposited on the susceptible cultivar CMC 40. Antibiosis is expressed by mites having lower fecundity, a longer development time, a shorter adult life span, and higher larval and nymphal mortality when feeding on resistant vs. susceptible cultivars.

Host plant resistance studies for *Tetranychus* mite species have been less intensive than those carried out for the CGM. Field evaluations of cassava germplasm by CIAT in Colombia have been hindered by low *Tetranychus* mite field populations. However, some laboratory studies have been conducted. *T. urticae* larval and nymphal mortality was 68% higher on the cultivar MBra 12 than on the susceptible cultivar MCol 22. Mortality on the cultivar MCol 1434 was 50% higher than that on MCol 22. Egg eclosion and larval survival was significantly lower (25%) on MCol 1351 than on MCol 22. Germplasm evaluations for resistance to *Tetranychus* mite need to be carried out in regions where high mite populations occur. The opportunity for this line of research is better suited for those regions of Asia where *Tetranychus* mite species are reported to cause economic damage.

Several wild *Manihot* species have been evaluated as a potential source of resistance to cassava mites by CIAT in Colombia. Moderate levels of resistance (2.0 on a 1.0 to 6.0 damage scale) to *M. tanajoa* have been found on some accessions of *M. esculenta* subspp. *flabellifolia*. In laboratory studies, *M. tanajoa* oviposition was reduced considerably on some accessions of *M. tristi*, *M. filamentosa* and *M. alutacea*, when compared to a susceptible *M. esculenta* (CMC 40) cultivar. These results indicate that a research effort to utilize wild *Manihot* species as a source of resistance to cassava mites is needed. This effort could also be extended to include *Tetranychus* mite species.

Biological control

Biological control offers an alternative and a practical solution for management of cassava mites, provided that chemical pesticides are not being employed to control other arthropod pests, such as whiteflies and mealybugs.

Beginning in the early 1980s, extensive evaluations of the natural enemy complex associated with cassava mites were conducted at more than 2,400 sites in 14 countries of the Neotropics (Bellotti *et al*., 1987). The primary target in most of these field and lab studies was the CGM. Predator species feeding on *Tetranychus* mite species were also collected and evaluated. These ongoing extensive surveys indicate that the CGM is present throughout much of the lowland Neotropics; high populations, causing significant yield loss can be localized, occurring most frequently in Northeast Brazil.

Geographic regions of the Americas were identified and prioritized using GIS support, to assist in targeting specific areas for exploration. Homologous maps based on agrometeorological data and microregional classification comparing Africa and the Neotropics were prepared as one of the major targets for biological control in those areas of Africa where the CGM was causing economic damage.

A total of 87 phytoseiid species were collected and stored: 25 are new or unrecorded species; 66 were collected from cassava (**Table 4**). The current predator mite reference collection held at CIAT conserves primarily those related to phytophagous mites found on cassava. A taxonomic key on the species associated with cassava is being prepared with Brazilian colleagues. The CIAT-Brazil collection is a true reference collection with accompanying database and can be readily used for species description. Explorations also identified several insect predators of cassava mites, especially the staphylinid *Oligota minuta* and the coccinellid *Stethorus* sp. After extensive lab and field studies of this predator complex, it was generally agreed that the phytoseiid predators offer the best potential for controlling mites, especially when occurring in low densities. The phytoseiid development cycle is shorter than that of the CGM and *Tetranychus* mites. In studies at CIAT with the species *Neoseiulus anonymus*, the egg-to-adult development period at 25 and 30°C was 4.7 and 4.0 days, respectively (Bellotti, 2002). This is approximately half the development period for the CGM and *T. urticae* at those temperatures. Survey data also revealed that CGM densities were much higher in Northeast Brazil than in Colombia, but the richness of phytoseiid species was greater in Colombia.

Field data from experiments in Colombia demonstrated that a rich phytoseiid species complex could reduce CGM populations and prevent cassava yield loss. When natural enemies were eliminated by applying low doses of an acaricide that did not affect the CGM population, cassava root yields were reduced by 33%. Application of an acaricide did not increase yields, indicating the effectiveness of biological control (Braun *et al*., 1989).

A major objective of the surveys for CGM natural enemies and the substantial research that followed was to identify the key phytoseiid species controlling CGM populations and introduce them into Africa. This was a collaborative effort between CIAT and EMBRAPA in the Americas and IITA in Africa. Of the phytoseiid species identified as feeding on CGM, those most frequently collected were *Typhlodromalus manihoti* (found in >50% of the fields surveyed), *Neoseiulus idaeus, Typhlodromalus aripo, Galendromus annectens, Euseius concordis* and *Euseius ho* (Bellotti *et al*., 1987).

Table 4. Natural enemies (insects, mites/phytoseiidae and entomopathogens) of mites feeding on cassava.

Principal Species	*Predators*	*Entomopathogens*
Mononychellus tanajoa	**Insects:**	
	Stethorus tridens	*Hirsutella thompsoni*
	S. darwin	*Neozygites floridana*
	S. madecasus	*N. tanajoae*
	Oligota minuta	
	O. gilvifrons	
	O. centralis	
	O. pigmaea	
	Delphastus argentinicus,	
	Chrysopa sp.	
	Mites/Phytoseiidae*:*	
	Typhlodromalus manihoti	
	T. aripo	
	T. rapax	
	E. lokole	
	E. ncholsi	
	E. baetae,	
	Neoseiulus idaeus	
	Galendromus annectes	
	Euseius concordis	
	Euseius ho	
	Euseius fustis (5)	
Tetranychus canadensis	*Euseius naindaimei*	
	Amblyseius aerialis	
	Galendromus annectens	
	Typhlodromalus manihoti	
	T. rapax	
	Phytoseiulus macropilis	
	Phytoseius purseglovi	
	Typhlodromips dentilis	
Tetranychus tumidus	*Amblyseius chiapensis*	
	A. largoensis	
	A. aerealis	
	Euseius naindaimei	
	Galendromus annectens	
	Neoseiulus anonymus	
	Phytoseiulus macropilis	
	Typhlodromalus manihoti	
	T. dentilis	

Table 4. Continued

Principal Species	*Predators*	*Entomopathogens*
	T.aripo	
	T.rapax	
	Typhlodromips bellotti	
Tetranychus marianae	*Cydnodromella pillosa*	
T. mexicanus	*E useius casaeriae*	
	Galendromus annectens	
	Neoseilus anonymus	
	Phytoseiulus macropilis	
	Typhlodromalus peregrinus	
Tetranychus yusti	*Euseius ho*	
	Neoseiulus idaeus	
	Neoseilus anonymus	
	Phytoseiulus macropilis	
	Typhlodromalus manihoti	
	T. tenuiscutus	
Tetranychus kanzawai	*Neoseiulus longispinosus*	
Tetranychus urticae	*Neoseilus anonymus*	
	Galendromus helveolus	
	Phytoseiulus macropilis	
	Typhlodromalus aripo	
	T. manihoti	
	T. rapax	
Tetranychus bastosi	*Neoseiulus idaeus*	
	Neoseilus anonymus	

More than ten species of phytoseiids were shipped from Colombia and Brazil to Africa, via quarantine in England (IIBC-International Institute of Biological Control). None of the Colombian species became established, but three of the Brazilian species did (*T. manihoti*, *T. aripo* and *N. idaeus*). *T. aripo*, the most successful of the three species, was released in Africa in 1993 and is found in more than 14 countries (Yaninek *et al*., 1993). *T. aripo* inhabits the apex of cassava plants during the day and forages on leaves at night and can persist during periods of low CGM densities by consuming alterative food sources (e.g., maize pollen). On-farm trials in Africa indicate that *T. aripo* reduced CGM populations by 30-90% and increased fresh root yields by 30-37%. This represents an increase of US$ 60/ha for cassava producers (Yaninek *et al*., 1993; Onzo *et al*., 2005).

Numerous predator mite species (Phytoseiidae) have been observed feeding on *Tetranychus* mite species and several species have been evaluated feeding on *T. urticae*. These include *G. annecteus*, *E. concordes*, *Phytoseilus pessimilis*, *Neoseiulus anonymous*, *N. chilenensis*, *N. idaeus* and *P. macropilis*. The development time (egg to adult) for the seven species ranged from 4.2 days (*N. idaeus*) to 6.1 days (*G. annecteus*) (25ºC, 70±5% RH and 12:12 hours photoperiod) when feeding on *T. urticae*. The development time for these seven species when feeding on *M. tanajoa* ranged from 4.0 (*P. pesimilis*) to 5.8 days (*G. annecteus*). The development time during these experiments for the phytophagous species, *T. urticae* and *M. tanajoa,* was 9.1 and 10.7 days, respectively. The development time of

three predator species, *N. chilenensis* (4.4 days), *P. macropilis* (4.3 days) and *N. idaeus* (4.2 days) was less than one-half that of *T. urticae* (9.1 days). *N. anonymous* development time when feeding on *T. urticae* was 4.7 days, nearly one-half that of the prey species.

Adult longevity of the phytoseiid species when feeding on *T. urticae* ranged from 17.4 days (*N. idaeus*) to 54.8 days (*N. chilenensis*). When feeding on *M. tanajoa*, adult longevity ranged from 18.2 days (*N. ideaus*) to 44.1 days (*P. macropilis*). In general, the data for both species was similar.

Predation consumption studies were carried out with the phytoseiid *N. anonymous* on both prey species. *N. anonymous* consumed all four-development stages (eggs, larvae, nymphs and adults) of *T. urticae* and *M. tanajoa*. *N. anonymous* when feeding on *T. urticae* consumed an average of 78.3 eggs, compared to only 10.3 *M. tanajoa* eggs. However, *N. anonymous* showed a higher average consumption of larvae (20.0 vs. 2.8), nymphs (9.0 vs. 5.1) and adults (21.0 vs. 4.7) when feeding on *M. tanajoa* compared to *T. urticae*. These results indicate that *N. anonymous* could play an effective role in the biological control of both prey species, especially that of *T. urticae* because of the high egg consumption.

Neozygites sp. is a fungal pathogen (Zygomycetes: Entomophthorales) found on mites throughout cassava-growing regions of the Neotropics. Isolates of *Neozygites floridana* from Brazil and Colombia, and from *M. tanajoa* from Brazil and Benin were evaluated on the CGM in Africa. Laboratory and field studies indicate that the Brazilian strain of *N. floridana* was the most virulent. Although this fungus shows considerable promise for biological control of the CGM, further research and field evaluations are needed (Delalibera *et al*., 1992).

Exotic phytoseiid mite predators can play an important role in reducing CGM populations in Africa and *Tetranychus spp*. in Asia. However, field observations in the Neotropics indicate that they are very sensitive to disturbances in the agro-ecosystems, especially the use of pesticides. For example, when insecticides were applied at CIAT for controlling thrips, CGM populations erupted, and few phytoseiid predators were detected in the fields. Studies in Colombia showed that low acaricide doses that did not cause mortality to CGM, were lethal to phytoseiids, causing a considerable increase in mite populations and cassava yield losses (Braun *et al*., 1989). In the Neotropics, especially on larger plantations, cassava farmers may use pesticides to control hornworm, whitefly or thrips outbreaks. This could result in mite outbreaks and yield losses if biological control is the only control measure employed, and highly susceptible cultivars are being grown.

CIAT maintains a cassava phytophagous mite and a phytoseiid predator mite collection that has been sourced from many of the cassava growing regions of the Americas, Asia and Africa. The collection contains more than 20,000 specimens and is utilized by cassava scientists for comparative taxonomic purposes. The collection is accompanied by a computerized database that allows researchers to identify the geographic location of cassava mite species and their natural enemies. It also allows us to predict the climatic parameters that might favor outbreaks or spread of phytophagous mite species and the potential adaptability of their natural enemies.

e. Recommendations for control of cassava mites

1. Stake treatment (Thaimethoxam) in endemic areas.
2. Plant at the beginning of the rainy season (to guarantee good establishment).
3. Appropriate fertilization to improve plant vigor.
4. Use resistant or tolerant cultivars where available.
5. Water sprayed under pressure will reduce mite populations.
6. Only use selective insecticides to protect natural biological control.
7. Phytoseiid mite predators are very sensitive to pesticides (even low dose applications).
8. Quarantine measures (prevent invasive species).

4. Thrips

a. Taxonomy

Several species of thrips are reported feeding on cassava, primarily in the Americas. The most important include *Frankliniella williamsi, Corynothrips stenopterus, Scirtothrips manihoti, Caliothrips masculinus* and *Scolothrips* sp. More recently, a new species of thrips (*Thrichinothrips strasser*) associated with cassava was reported from Costa Rica. *F. williamsi* is reported feeding on cassava in Africa, and high populations of *S. manihoti* have recently been reported from central Brazil. Four thrips species have been reported feeding on cassava in Asia; *F. williamsi, Ayyaria Chetophora, Elaphrothrips denticollis* and *Nesothrips lativentris. F. williamsi* appears to be the most important species and the only one reported causing yield losses **(Table 1)** (Bellotti, 2002; 2008).

b. Damage

F. williamsi larvae and adults feed on the growing points and young leaves of cassava, which do not develop normally; leaflets are deformed and show irregular chlorotic spots. The rasping-sucking stylet-like mouthparts damage leaf cells during expansion, causing deformation and distortion and parts of the leaf lobes are missing. Brown wound tissue appears on the stems and petioles, and internodes are shortened. Growing points may die, causing growth of lateral buds, which may also be attacked, giving the plant a witches'-broom appearance that can be confused with viral disease symptoms.

Yield reductions induced by *F. williamsi* range from 5-28%, depending on varietal susceptibility. The average reduction for eight varieties in Colombia was 17.2%. Thrips damage and yield reduction are especially pronounced in the seasonally dry tropics where the dry season is at least three months. Plants recover with the onset of the rainy season (Bellotti, 2002).

c. Management

F. williamsi is not considered a major pest of cassava as it is not often reported causing yield losses in farmers' fields. It can be controlled easily by using resistant pubescent cultivars. Approximately 50% of the CIAT cassava germplasm bank is pubescent, and resistant to *F. williamsi*. Resistance is based on leaf bud pilosity, and increasing pubescence of unexpanded leaves increases thrips resistance. Observations indicate that most landrace varieties grown by farmers in the seasonally dry lowland neotropics are pubescent. It is hypothesized that cassava growers may have selected pubescent varieties over time for the absence of thrips damage.

Biological control of thrips on cassava has not been studied in detail. However, mite predator species (*Phytoseiidae)*, such as *Typhlodromalus aripo* and the insect predator *Orius* sp *(Hemiptera: Anthocoridae)* have been observed feeding on cassava thrips **(Table 5)**.

Table 5. Natural enemies (parasitoids, predators and entomopathogens) of other important cassava pests.

Principal Species	Parasitoids	Predators	Entomopathogens
***Frankliniella williamsi* (thrips)**		*Orius sp.*	
***Scirtothrips manihoti* (thrips)**		*T. aripo*	
***Vatiga illudens* (lacebug)**		*Zelus sp.*	
***Vatiga manihotae* (lacebug)**		*Zelus nugax*	
***Erinnyis ello* (hornworm)**	*Trichogramma spp.* *Telenomus sphingis* *Cotesia americana* *Cotesia sp.* *Euplectrus sp.* *Drino macarensis* *Drino sp.* *Euphorocera sp.* *Sarcodexia innota* *Thysanomia sp.* *Belvosia sp.* *Forciphomyia eriophora* *Cryptophion sp.* *Ooencyrtus sp.* *Chetogena scutellaris*	*Chrysopa spp.* *Podisus nigrispinus* *P. obscurus* *Polistes carnifex* *P. erythrocephalus* *P. versicolor* *P. canadensis* *Polybia emaciata* *P. sericea* *Zelus nugax* *Zelus sp.* *Calosoma sp.* *Dolichoderus sp. ,* *Alcaeorrhynchus grandis* **Spiders**: Tomicidae, Salticidae	*Bacillus thuringiensis* *Baculovirus of E. ello* *Metarhizium anisopliae* *Beauveria bassiana* *Paecylomices sp.* *Nomuraea rileyi* *Cordyceps sp.*
***Erinnyis alope* (hornworm)**	*Trichogramma spp.* *Telenomus sp.*	**Spiders**: Tomicidae, Salticidae. *Chrysopa sp.*	
***Phoenicoprocta sanguinea* (tiger moth)**	*Cotesia sp.* *Euplectrus sp.* *Trichogramma spp.*		
***Chilomima clarkei* (stemborer)**	*Brachymeria sp.* *Tetrastichus howardi* *Trichogramma sp.*		
***Aonidomytilus albus* (scale insect)**	*Aphytis diaspidis* *Aphytis lignanensis* *Encarsia aurantii* *Azotus sp.*	*Prodilis sp.* *Cryptognatha auriculata* *Azya sp.*	
***Saissetia miranda* (scale insect)**	*Anagyrus sp.* *Metaphycus sp.* *Scutellista cyanea*		

Table 5. Continued

Principal Species	Parasitoids	Predators	Entomopathogens
Iatrophobia brasiliensis (gall midge)	*Torymoides sulcius*		
Anastrepha pickeli (fruitfly)	*Opius sp.*		
Anastrepha manihoti (fruitfly)	*Opius sp.*		
Cyrtomenus bergi (burrower bug)		*Nerthra sp.*	*Heterorhabditis sp* *Metarhizium anisopliae* *Steinernema spp* *Heterorhabditis bacteriophora* *Metarhizium anisopliae* *Beauveria bassiana* *Paecilomyces lilacinus*
Phyllophaga menetriesi (white grub)		*Campsomeris dorsata*	*Metarhizium anisopliae* *Beauveria bassiana* *Heterorhabditis bacteriophora* *Steinernema feltiae*
Stictococcus vayssierei (root mealybug)		*Anoplolepis tenella*	
Zonocerus elegans (grasshopper)			*Metarhizium anisopliae* *Beauveria bassiana*
Zonocerus variegates (grasshopper)			*Metarhizium anisopliae* *Beauveria bassiana*

5. Cassava lacebugs

a. Taxonomy

Reported as pests of cassava only in the Neotropics, five species of the genus *Vatiga* show a decided preference for feeding on cassava: *Vatiga illudens, V. manihotae*, *V. pauxilla, V. varianta* and *V. cassiae* (Heteroptera: Tingidae) **(Table 1)**. The first two are the most widely distributed and the most damaging to cassava. *V. illudens* predominates in Brazil but also occurs throughout the Caribbean region and may be present in other areas. *V. manihotae*, the most widespread lacebug, is consistently found on cassava in Colombia and Venezuela, but is also reported from Cuba, Trinidad, Peru, Ecuador, Paraguay, Argentina and Brazil. *Vatiga* spp. has also been reported feeding on wild species of *Manihot*. In 1985, the

black lacebug, *Amblystira machalana*, was first observed causing damage to cassava in different regions of Colombia, Venezuela and Ecuador.

b. Damage

Lacebug adults and nymphs feed on the undersurface of lower and intermediate leaves, but can also damage upper leaves. Feeding by *Vatiga* spp. causes leaves to form yellow spots that eventually turn reddish brown, resembling *Tetranychus* mite damage. *A. machalana* feeding is characterized by white feeding spots that increase in area until leaf centers turn white and eventually darken. High lacebug populations will cause leaves to curl and die, often resulting in defoliation of lower leaves. Higher populations are observed on younger plants (4-5 months) but decline as plants age (Bellotti, 2008).

The relationship between damage and population density and duration is not entirely understood. In recent field trials with natural populations of *A. machalana* at CIAT, yield losses ranged from 8.1-42.7%, depending on cultivar susceptibility and duration of lacebug attack.

Populations of *V. illudens* in Brazil are endemic and appear to be causing yield losses, especially in the central Campo Cerrado regions, although high populations are also reported from the South and Northeast. In the Campo Cerrado region, root yield losses of 21% have been reported. Lacebug (*V. illudens*) damage to cassava in Southern Brazil (Parana, Sao Paulo and Mato Grosso do Sur) has increased in recent years and cassava producers are spraying pesticides for control. These higher populations in the region may be due to higher temperatures or lengthening of the dry season.

c. Biology and behavior

Prolonged dry periods favor high populations of *V. illudens* and *V. manihotae*. In contrast *A. machalana* attack can occur during both wet and dry seasons, but is more likely during rainy periods. Observations in Colombia indicate shifts in lacebug populations. *V. manihotae* was the predominant species in the Cauca Valley until the mid-1980s. By 1990 *A. machalana* populations dominated. More recently *V. manihotae* increased and is once again the predominant species, while *A. machalana* is difficult to find. The cause for this shift in populations is unknown. In Ecuador, populations of *A. machalana* remain high.

The egg stage of *V. manihotae* is 8-15 days followed by five nymphal instars averaging 16-17 days; adult longevity was 40 days under field conditions. Laboratory studies with *V. illudens* in Brazil reported a nymphal duration of 13.5 days and an average adult longevity of 27 days. In lab studies with *A. machalana*, the egg stage averaged 8.2 days; the five nymphal instars, 14 days; average adult longevity, 22 days.

d. Management

Lacebugs are the least studied of the important cassava pests so considerable research is required before sound and efficient management practices can be recommended. These studies should be conducted in Brazil where *V. illudens* is endemic. Lacebug control appears difficult as few natural enemies have been identified, and chemical control should be avoided. In Colombia and Ecuador, observations indicate that *V. manihotae* or *A. machalana* populations are not high enough to warrant pesticide applications. Preliminary screening of cassava germplasm in Brazil and Colombia indicates that host plant resistance may be

available, but no germplasm development program is attempting to develop resistant cultivars. In insectary studies in Brazil using caged *V. illudens*-infested plants, isolates of the fungal entomopathogens *Metarhizium anisopliae* and *Beauveria bassiana* caused 100% and 74% mortality of the lacebugs, respectively, indicating the potential of these fungi for lacebug control.

6. Cassava hornworms

a. Taxonomy

Several lepidopterans feed on cassava, the most important being the cassava hornworm, *Erinnyis ello*, which causes serious damage to cassava in the Neotropics and has a broad geographic range, extending from southern Brazil, Argentina and Paraguay to the Caribbean basin and southern USA. The migratory flight capacity of *E. ello*, its broad climatic adaptation and wide host range probably account for its wide distribution. Several other species of *Erinnyis* (*E. alope*; and subspecies *E. ello ello, E. ello encantado*) are reported feeding on cassava in the Neotropics, but they appear to be of minor importance and do not cause economic damage to the crop.

b. Damage

Hornworm larvae feed on cassava leaves of all ages, and high populations will also consume young, tender stems and leaf buds. Severe attacks cause complete plant defoliation, bulk root loss and poor root quality. In farmers' fields, natural attacks resulted in 18% yield loss; simulated damage studies resulted in 0-64% root yield loss, depending on number of attacks, plant age and edaphic conditions. Repeated attacks are more common when poorly timed pesticide applications fail to destroy fifth instar larvae or prepupae. Frequent attacks often occur on larger plantations (>100 ha), where subsequent populations can oviposit and feed on areas not previously defoliated. Severe attacks and complete defoliation do not kill cassava as carbohydrates stored in the roots enable recovery, especially during the rainy season. However, severe defoliation can reduce the dry matter and starch quality of cassava roots.

c. Biology and behavior

Although hornworm outbreaks are sporadic, they mostly occur during the rainy season when foliage is abundant. The grey nocturnal, migratory adult moths have strong flight abilities. *E. ello* females oviposit small, round, light green-to-yellow eggs, individually on the upper surface of cassava leaves. In field cage studies, females oviposited an average of 450 eggs, although as many as 1,850 eggs/female were observed. This high oviposition, combined with the mass migratory behavior of adults, helps explain the rapid buildup of hornworm populations and their sporadic occurrence. During the larval period, each hornworm consumes about 1,100 cm^2 of foliage; ca. 75% of this during the fifth instar. At 15, 20, 25 and 30ºC, the mean duration of the larval stage is 105, 52, 29 and 23 days, respectively, indicating that their peak activity may occur at lower altitudes or during the summer in the subtropics. When considerable leaf area is present, up to 600 eggs may be found on a single plant, and larval populations may exceed 100/plant. It is estimated that 13 fifth instar larvae can defoliate a 3-mo-old plant in 3-4 days, especially on low fertility soils. Given the foregoing, hornworm outbreaks must be controlled when populations are in the early larval stages (Bellotti, 2002).

d. Management

The migratory behavior of hornworm adults makes effective control difficult to achieve and reduces the impact of natural biological control. Insect migration has been described as an evolved adaptation for survival and reproduction, and some researchers speculate that the hornworm's migration evolved as a mechanism to survive low food availability, unfavorable environmental conditions and attack by natural enemies. It is important to detect hornworm outbreaks while in the early development stages. Successful control requires monitoring field populations to detect migrating adults, oviposition or larvae in the early instars. This can be done with black light traps for adults or by scouting fields for the presence of eggs and larvae (Braun *et al*., 1993).

Pesticides give adequate control if applied when hornworm populations in the early larval instar stages are detected and treated. Larval populations in the fourth and fifth instars are difficult to control. Farmers often react only when considerable defoliation has occurred, with excessive, ill-timed costly applications that can lead to repeated or more severe attacks. Pesticide use may also disrupt natural enemy populations, leading to more frequent attacks, a common occurrence on larger plantations.

More than 30 species of parasites, predators and pathogens of the egg, larval and pupal stages have been identified and reviewed extensively **(Table 5)**; however, their effectiveness is limited, most likely due to the migratory behavior of hornworm adults. Eight microhymenopteran species of the families Trichogrammatidae, Scelionidae and Encryrtidae are egg parasites, of which *Trichogramma* and *Telenomus* are the most important. In recent field surveys during a hornworm outbreak at CIAT, egg parasitism reached 68%; 57% due to *Trichogramma* sp. and 11% to *Telenomus* sp. Tachinid flies are important dipteran larval parasitoids and the Braconidae, especially *Cotesia* spp., are the most important hymenopteran. *Chrysopa* spp. are common egg predators, while important larval predators include *Polistes* spp. (Hymenoptera: Vespidae) and several spider species. Important entomopathogens include *Cordyceps* sp. (Aconycites: Clavicipitaceae), a soil-borne fungus that invades hornworm pupae, causing mortality. Recent lab studies show that certain isolates of *Beauveria* sp. and *Metarhizium* sp. cause high larval mortality. Hornworm outbreaks can be controlled with timely (early instars) applications of commercial biopesticides of *Bacillus thuringiensis* (Bellotti, 2008).

The effectiveness of biological control agents in a hornworm management strategy depends on the ability to synchronize the release of large numbers of predators or parasitoids to augment natural biological control. Predator and parasitic effectiveness in hornworm control is limited by poor functional response during outbreaks, which are of short duration (15 days). In the absence of a reliable commercial source of *Trichogramma* or other parasitoid or predator species, the cost of maintaining these natural enemies in continuous culture to guarantee availability when an *Erinnyis ello* outbreak occurs is economically prohibitive and impractical for most cassava farmers (Braun *et al*., 1993).

The complexities of inundatory releases of parasitoid and predator species suggest the need for a cheap, storable biological pesticide. A granulosis virus of the family Baculoviridae was found attacking *E. ello* in cassava fields at CIAT in the early 1970s. Pathogenicity studies using virus material extracted from infected larvae collected in the field were carried out on cassava plants in the lab and field. Larval mortality reached 100% 72

hours after application. Studies on the effect of virus concentration on mortality of larval instars showed a sigmoidal relationship for the first, second and forth instars. LD_{50} studies show that progressively higher concentrations are needed for adequate control of each succeeding larval instar. Most fifth instar larvae reached the prepupal stage, but few female adults emerged and those that did had wing deformities and died without producing progeny (Bellotti *et al*., 1992).

Although the baculovirus can be managed by small farmers, this technology has been most successful with larger producers or where research and extension services have provided access to it. Growers can collect and macerate diseased larvae and apply the virus suspension to cassava fields. The virus can be stored for several years under refrigeration, and for a few months at room temperature. Hornworm management with the baculovirus was implemented in southern Brazil during the late 1980s and early 1990s. Researchers and extension workers trained farmers in the handling and use of the virus and distributed free samples. By 1991, the virus was being applied on about 34,000 ha in Parana State at a cost of only about US$ 1/ha. In Santa Catarina state, virus applications to early instars resulted in almost complete control, and pesticide applications were reduced by 60%. The virus is at present being used to control the hornworm on large cassava plantations in southern Brazil.

In Venezuela, where the hornworm is endemic, the virus preparation was applied (70 ml/ha) to large cassava plantations (7,000 ha) via overhead sprinkler irrigation systems when larvae were in the first and second instars. This not only resulted in 100% control but also eliminated pesticides; the cost of gathering, processing, storing and applying the virus preparation was only US$ 4/ha.

In Colombia, a baculovirus biopesticide was developed by a private company (Biotropical) in collaboration with CIAT. The product has been approved for commercial release by MADR and is available as a wettable powder. Field trials to evaluate the efficacy of this product (Bio Virus Yuca®) were carried out in two locations of Colombia: the Provinces of Tolima and Risaralda. During natural hornworm attacks, the baculovirus applications (300 g/ha) resulted in 93% hornworm mortality in Tolima and 85% in Risaralda.

The key to effective hornworm control is training farmers to detect outbreaks through light trapping of adults or field monitoring combined with the timely application of a biopesticide (or chemical insecticide) when larvae are in their early instars (1-3).

B. Stemborers and Stem Feeders

Numerous insect species can feed on and damage cassava stems and branches. Although some species are nearly worldwide in distribution, the most important are in the Neotropics. Four pests will be discussed in this section: stemborers, scale insects, fruitflies and shootflies. Several other pests can also damage the stem (e.g., mites, thrips, mealybugs, hornworms and grasshoppers); however, they are primarily leaf feeders and are discussed elsewhere. Dipteran fruitflies (*Anastrepha* spp.) and shootflies (*Neosilba* sp.) can also bore into the stem and are discussed here.

Stemborers can damage cassava in two ways: They can (i) weaken the plant by tunneling in the stems, causing breakage that will reduce yields; and (ii) destroy or reduce the quality of stem cuttings, thereby affecting germination and vigor of the planting material.

7. Stemborers

a. Taxonomy

A complex of arthropod stemborers that includes both lepidopteron and coleopteran species feed on and damage cassava. In the Neotropics, stemborers are most important in Colombia, Venezuela and Brazil. Seven species of *Coelosternus* (Coleoptera: Curculionidae) can reduce cassava yields and quality of planting material in Brazil; however, the damage is generally sporadic and localized, and significant yield losses are not reported. The species *Lagochirus aranciformes* (*Coleoptera: Cerambycidae)* damages cassava in Colombia, causing stem breakage and a loss in planting material. *Lagochirus* sp. is reported feeding on cassava stems in Africa and Asia but there are no reports of yields losses. *Dorysthenes buqueti (Coleoptera: Cerambycidae*) is reported damaging cassava stems in Thailand **(Table 1)**.

Populations of the lepidopteron stemborer *Chilomima clarkei* (Fam. Pyralidae) have increased dramatically in Colombia and Venezuela in recent years, to the point where it is now considered an important pest of cassava, causing yield losses and damage to stem cuttings. On the Atlantic Coast of Colombia (Provinces of Magdalena and Cesar), *C. clarkei* damage was detected in 85% of the cassava plantations surveyed (Bellotti, 2002; 2008).

b. Damage

C. clarkei populations can occur throughout the year but are higher during the rainy season. From 4-6 overlapping cycles can occur during the 1-year crop cycle, increasing potential damage and making control more difficult. Stem breakage can occur when there is extensive tunneling by larvae. When over 35% of the plants suffer stem breakage, yield losses range from 45-62%. Larval tunneling can also lead to stem rot and a reduction in the quantity and quality of planting material. Attacks are easily detected by the presence of excreta, sawdust and exudates ejected from burrows made in infested stems.

Larvae of the *Coelosternus* weevils damage cassava by penetrating the stem and tunneling into the center or pith region. This weakens the plant and stems and branches may eventually dry and break, reducing the quantity and quality of planting material. When the attack occurs around the base of the plant stem breakage and lodging may occur. C. *sulcolutus* larvae have been observed feeding on the underground parts of the stem but have not been found attacking roots.

Lagochirus larvae (long-horned beetles) can cause damage similar to that of *Coelosternus*. Damage is usually at the base of the plant and strong winds will often result in stem breakage. During dry periods affected branches may desiccate resulting in defoliation. *Lagochirus* attacks are characterized by considerable excreta, sawdust and exudate around the base of the plant. Stem borer larvae can be found in the tunnels at the site of infestation or on the ground beneath the plant. Heavy infestations may cause plant mortality.

c. Biology and behavior

Adult *C. clarkei* females are nocturnal moths and oviposit in cassava stems, usually around the bud or node. The tan-colored females can oviposit more than 200 eggs in a 5-6 day period. The egg stage averages 6 days (28°C). The highly mobile first instar larvae feed on the outer bark or stem epidermis. Upon finding an appropriate feeding site, usually around lateral buds, the larvae form a protective web, under which the first four instars feed, enlarging the web with each instar. Stem penetration occurs during the fifth instar larval stage. Extensive tunneling can occur as the larval cycle is completed (6-12 instars). Pupation occurs in the stem and winged adults emerge. The larval stage is 32-64 days, followed by the pupal stage (12-17 days). Female adults live 5-6 days; males, 4-5.

Coelosternus females may oviposit on various parts of the plant beneath the bark or near broken or cut ends of branches. Some species prefer the tender parts of the plant. Females may deposit several white eggs, but often no more than one per day. Larvae may vary in size depending on the species. Fully grown larvae of *C. alternas* are 16 mm in length and 4 mm wide, while *C. tarpides* larvae are 9.0 x 2.5 mm. Most larvae are curved with a yellowish white to pale brown body a reddish brown head capsule and black mandibles. Several larvae may be found in one stem. The larval period ranges from 30-60 days and the fully-grown larvae of all species pupate within a cell constructed in the pith region. The pupal period is about one month. Adults are light to dark brown, often covered with yellowish scales and range in length from 6 mm (*C. granicollin)* to 12 mm (*C. alternans* and *C. rugicollis*), and may be active throughout the year. Higher *Coelosternus populations* are often associated with older plants that have been left in or around the fields. These populations from older plants probably provide weevil populations that attack younger plants (Farias and Bellotti, 2006).

Lagochirus adults oviposit in stems and branches about 2.5 cm below the bark and eggs hatch in 5 to 6 days. The larvae, which may take up to two months to develop, can measure up to 29 mm. They generally feed around the base of the plant and several may be found in one plant. The pupal period is about one month and occurs within the stem. Adults are nocturnal, rapid flyers and active throughout the year. They are brown in color, about 17 mm in length and may feed on leaves and bark (Bellotti, 2002).

d. Management

Stemborer control is difficult once the larvae enter the stem and tunneling begins. In addition, the web formed by the early larval stages of *C. clarkei* acts as a protective devise against natural enemies and pesticide applications. The mobile first instar larvae are vulnerable and more exposed to both natural enemies and pesticides. Biopesticides such as *Bacillus thuringiensis* (*Bt*) are recommended; however, with overlapping generations, several applications may be required, which would be too costly for small producers. Intercropping with maize will reduce *C. clarkei* populations, but only until the intercrop is harvested.

Natural enemies such as hymenopteran parasitoids (*Apanteles* sp., *Brachymeria* sp., *Tetrastichus howardi* and *Trichogramma* sp.) have been identified **(Table 5)**, but their role in regulating stemborer populations has not been investigated. The fungal entomopathogens *Metarhizium anisopliae* and *Beauveria bassiana* have been identified as possible biological control agents.

CIAT has worked on identifying cassava germplasm resistant to *C. clarkei*. More than 1,000 genotypes have been evaluated on the Colombian Caribbean Coast, where *C. clarkei* populations are consistently high. Evaluations are based on the number of holes and tunnels and percent stem breakage. Genotypes with 0-1 holes/stem indicate varietal influence and the need for further evaluation. As natural field populations of *C. clarkei* are used in these evaluations, results may be misleading because genotypes exhibiting low infestation may be 'escapes' (i.e., have avoided damage by chance). CIAT has initiated research to introduce insect-resistant Bt genes through *Agrobacterium*-mediated transformation into cassava embryonic tissue to develop resistant cultivars.

Cultural practices such as selection of clean cuttings and burning plant residues especially stems and branches, are recommended for reducing stemborer populations. Older cassava plants (2 or more years) should also be destroyed, as they are often a source of stemborer infestation. Control with pesticides is impractical as it is difficult to kill the larvae once they are within the stem. Natural enemies of *Lagochirus* and *Coelosternus* species have not been recorded. Intercropping with maize was shown to reduce *C. clarkei* populations but only until the intercrop was harvested.

8. Scale insects

a. Taxonomy

Several species of scales are reported attacking cassava stems and leaves in the Americas, Africa and Asia. Although reductions in yield due to scale attack have been reported, they are not considered to be serious pests of cassava. The most important species are *Aonidonytilus albus* and *Saissetia miranda*. *A. albus* has been reported on cassava throughout most of the cassava-growing regions in the world and is considered the most widely distributed cassava pest. It is easily disseminated from one region to another through stem cuttings, which probably accounts for its wide distribution. The species *Parasaissetia nigra* is reported from Asia **(Table 1)**.

b. Damage

Aonidonytilus albus outbreaks are severer during the dry season. Their incidence increases when scale-infested stem cuttings are used for planting material. High *A. albus* populations may cover the stem and lateral buds. Leaves on heavily infested stems yellow, and defoliation can occur. With severe attacks the plants are stunted and stems can desiccate, leading to plant mortality. Some scale species can attack the leaves, but the greatest damage appears to be the loss of planting material. The germination of heavily infested cuttings is greatly reduced; and when they do germinate, the roots are poorly developed, reducing plant vigor. Yield losses of 19% were recorded at CIAT on plants heavily infested with *A. albus,* and there was a 50-60% loss in stake germination (Bellotti, 2002).

Scale infested stored stems or cuttings can also be lost, as scales will easily move between stems an increase rapidly in population.

c. Biology and behavior

The *A. albus* female scale is mussel shaped and covered with a white waxy excretion that acts as a protective covering. The young female nymphs are mobile for a short time, select a favorable location on the stem or leaves, insert their stylets and feed; females do not move for the remainders of their lives. Unlike the females, males have well developed legs

and wings. The female produces 40 to 50 eggs, depositing them between the upper scale covering and the lower cottony secretion. Eggs hatch in about 4 days; the first nymphal instars (crawlers) are locomotive and can disperse. When the crawlers become fixed (1 to 4 days) they cover themselves with numerous fine threads, molt in 11 days and become immobile. One female generation is from 22-25 days.

In laboratory studies at CIAT on excised cassava stems, male scales pass through two nymphal instars, averaging 10 and 6.5 days, respectively, and a prepupal and pupal stage of 4.5 days in total. Male adults live only 1-3 days and the male life cycle is about 23 days. *A. albus* females pass through three nymphal instars, averaging 10, 5 and 9 days respectively. The third instar is the adult stage. Nymphs emerge from eggs oviposited under the scale during a 7-day period, from the third to fifth day. Each female produced an average of 43 nymphs. Dispersal occurs by wind, active crawing or infested cuttings. The most important means of dissemination is by storing infested cuttings with healthy ones.

d. Management

The most effective means of control is through the use of clean, uninfested planting material and destroying infested plants to prevent the spread of infestation. Stem cuttings for vegetative propagation should be carefully selected from uninfested plants. The mussel-shaped *A. albus* grey-to-white female is difficult to detect, especially when populations are low and attached to stems around the lateral buds.

Treating stem cuttings that have originated from fields with scale attack is highly recommended. Dipping the cuttings in a pesticide emulsion for 5 min. is effective against light *A. albus* infestations. Heavily infested cuttings should not be sown as they will germinate poorly even if treated with a pesticide. If stems cut for propagation are obtained from infected fields they can be treated in an insecticidal dip of Malathion E.C. 57%, 1.5cc of the commercial product per liter of water.

9. Fruitflies

a. Taxonomy

Two species of fruitflies, *Anastrepha pickeli* and *Anastrepha manihoti* (Diptera: Tephritidae), whose origin is the Neotropics, are reported attacking cassava fruits from several regions of Central and South America. *Anastrepha montei* is reported infesting seed capsules in Costa Rica. Fruitflies are not reported attacking cassava in Africa, nor in Asia. Infestation of cassava fruits causes no economic damage and is of no concern to cassava producers. When oviposition occurs in the fruit, the larvae bore throughout the fruit, destroying the developing seed, which is a problem only for plant breeders.

b. Damage

Plant damage occurs when the tan-to-yellow colored females oviposit in the tender upper portion of the cassava stem in certain areas during the rainy season. The developing larvae become stemborers, tunneling into the apical stem, which provides an entrance for soft rot bacteria such as *Erwinia caratovora*, resulting in severe rotting of stem tissue and apical dieback. Several larvae may be found in one stem; their presence can be noted by the white liquid exudate that flows from their tunnel. Damage is severer on younger (2-5 months) plants. Nevertheless, the plants can recover from fruitfly damage. Yield losses have not been reported, but there is a reduction in the quality of stem cuttings for planting material. When

there is severe damage to the pith region of the stem, there is a reduction in germination. Yield losses can occur if severely damaged cuttings are used as planting material. It is therefore important that only stem cuttings without damage to the pith region be sown for vegetative propagation (Bellotti, 2002).

10. Shootflies

a. Taxonomy

Damage has been observed in most of the cassava-growing regions of the Americas but has not been reported from Africa or Asia. The most important species are *Silba pendula* and *Neosilba perezi* (Diptera :Lonchaeidae:). Severe attacks have been reported from Cuba, southern Brazil and parts of Central America, especially Costa Rica.

b. Damage

Larval feeding damage is manifested by a white-to-brown exudate flowing from cassava growing points, which eventually die. This breaks apical dominance, retards plant growth and causes germination of side buds, which leads to excessive branching. The dark metallic blue *S. pendula* adults deposit eggs in the growing points between the unexpanded leaves, and the young larvae tunnel in the soft tissue, eventually killing the apical bud. Attacks may occur throughout the year but are more prevalent at the onset of the rainy seasons and on recently germinated or young plants, resulting in a reduction in growth of the stems used for planting material. Yield is seldom affected (Bellotti, 2002; 2008).

c. Biology and behavior

S. pendula females have been observed ovipositing as many as 22 eggs per shoot, but 3-8 eggs per shoot is average. The eggs hatch in about four days and the young larvae tunnel in the soft, apical stem tissue. Several whitish larvae may be found in the affected tip. The larval exudates produced may provide a protection against parasites and insecticides. The larval period is about 23 days; larvae pupate in the soil and the adult fly emerges about 26 days later. Adults appear more active on sunny days.

d. Management

If plants are being grown for quality cuttings, the crop needs to be protected only during the first 3 months of growth. Usually one timely systemic pesticide application suffices to protect the crop. Pesticides, such as Dimethoate have been shown to provide adequate protection.

C. Soil-born Pests

The majority of the arthropod pests of cassava are ‘source’ pests, feeding on leaves and stems, which cause indirect damage by reducing root yield. Few are ‘sink’ pests, which cause direct, irrevocable damage to the edible roots. The most important and damaging root feeders appear to be generalists, and there is a hypothesis that cyanogenic potential in cassava is a defense mechanism against them. All cassava varieties have a high cyanogenic potential in leaves, stems and root peel. It can also be theorized that the root peel acts as a protective devise, especially in those varieties with low cyanogen levels in the root parenchyma. Three soil-borne pests are discussed here: the burrower bug, white grubs (several species) and root mealybugs.

11. Cassava burrower bugs

a. Taxonomy

First recorded as a pest of cassava in Colombia in 1980, *Cyrtomenus bergi* (Hemiptera-Heteroptera: Cydnidae), which appears to be native to the Neotropics, is a polyphagous feeder that attacks a wide range of crops and one of the few arthropod pests that feeds on the tuberous roots of cassava. Additional hosts include onions, peanuts, maize, potatoes, *Arachis pintoi* (forage peanuts), sorghum, sugarcane, coffee, asparagus, beans, peas, pastures and numerous weeds. It has also been reported feeding on cassava in Venezuela, Costa Rica, Panama and Brazil (states of São Paulo and Pará).

Cassava is not the optimal host for *C. bergi*. Fecundity, survival and intrinsic rate of population increase were highest on peanuts and forage peanuts, followed by maize. Sweet cassava, sorghum and onions were not favorable hosts, and *C. bergi* could not complete its life cycle on bitter cassava (Riis *et al*., 2005a).

b. Damage

C. bergi nymphs and adults feed on cassava roots by penetrating the peel and parenchyma with their strong thin stylet, leaving fine lesions in the plant tissue. This feeding action permits the entrance of several soil-borne pathogens (e.g., *Aspergillus, Diplodia, Fusarium, Genicularia, Phytophthora* and *Pythium* spp.*),* causing local rot spots on the parenchyma. The brown-to-black lesions begin to develop within 24 hours after feeding is initiated (Bellotti, 2008).

In cassava a quantitative scale to assess root damage was established, using a 1 to 5 rating based on the percentage of the parenchyma surface covered by rot lesions (1 = no damage, 2 = 1-25%, 3 = 26-50%, 4 = 51-75% and 5 = 75-100%). Studies show that even low *C. bergi* populations (close to zero) can cause more than 20% of the root to be covered with rot lesions. The darkened lesions on the white root parenchyma are not acceptable for the fresh consumption market; middlemen reject shipments of root with 20-30% damage, which translates into 100% loss for the farmers. Field trials in Colombia showed that damage can reach 70-80% of total roots, with more than a 50% reduction in starch content, thereby reducing the commercial value for the processing industry. As damage is not detected until roots are harvested and peeled, producers can lose the value of the crop as well as labor, time and land use.

c. Biology and behavior

C. bergi has five nymphal instars. It had a lifespan of 286-523 days when fed on slices of low-HCN cassava roots in the lab (23°C, 65± 5% RH). Egg eclosion averaged 13.5 days; mean development time of the five nymphal stages was 111 days; mean longevity for adults was 293 days (Bellotti and Riis, 1994).

C. bergi is strongly attracted to moist soils, and populations can occur in the soil throughout the crop cycle. It will migrate when soil moisture content is below 22% and is most persistent when it exceeds 31%. Thus, the rainy season greatly favors adult and nymphal survival, behavior and dispersal, whereas there is increased nymphal mortality during the dry season (Riis *et al*., 2005b).

Feeding preferences may be related to levels of cyanogenic glucosides in the cassava roots (Riis *et al*., 2003). Adults and nymphs that fed on high-HCN (>100 mg/kg) cultivars had longer nymphal development, reduced egg production and increased mortality. Oviposition on CMC 40 (43 mg HCN/kg) was 51 eggs/female versus only 1.3 on MCol 1684 (627 mg HCN/kg). Adult longevity on CMC 40 (235 days) was more than twice that on MCol 1684 (112 days). Additional studies indicate that the earliest instars are most susceptible to root cyanogenic potential (CNP). Due to the short length of the stylet, feeding during the first two instars is confined mainly to the root peel, whereas third to fifth instars can feed on the root parenchyma. CMC 40 has a low cyanogen level in the root parenchyma, but a high level in the root peel (707 mg HCN/kg). Feeding experiments in the lab resulted in 56% mortality of first and second instar nymphs feeding on CMC 40 and 82% for those feeding on MCol 1684. The high cyanogen level in the peel of CMC 40 is probably responsible for the high mortality (Bellotti and Riis, 1994).

Feeding preference studies carried out in the field in Colombia show that low-HCN cultivars suffer more damage than high-HCN ones. Three cassava varieties – MCol 1684 (high CNP), MMex 59 (intermediate CNP) and CMC 40 (low CNP) – were evaluated in field studies to determine the effect of CNP on *C. bergi* root damage. Ten months after planting, root damage on the low, intermediate and high CNP varieties was 85, 20 and 4%, respectively. These data indicate that high CNP may act as a feeding deterrent and that *C. bergi* should not be a problem where cassava with high CNP is cultivated (i.e. Northeast Brazil and many parts of Africa and Asia). However, in many cassava-producing regions, low CNP or 'sweet' varieties are preferred, especially for fresh consumption or starch markets.

d. Management

C. bergi can be the target of extensive chemical control, given the nature of the damage it causes to cassava as well as other crops. For example, in Colombia, control of *C. bergi* on crops such as onions, peanuts and coriander requires considerable pesticide use, with only marginal results. In cassava, pesticide use can reduce populations and damage; however, frequent applications may be required and they are costly and often fail to reduce damage below economic injury levels.

C. bergi control is difficult due to the polyphagous nature of the pest and its adaptation to the soil environment. As the initial damage can occur early in the crop cycle, control methods should be implemented either prior to or at planting, or during the first two months of crop growth. Intercropping cassava with *Crotalaria* sp. (sun hemp) reduced root damage to 4% versus 61% damage in cassava monoculture. This practice also reduced cassava yields by 22%; and as *Crotalaria* has little commercial value, this technology has not been readily adopted by producers (Bellotti, 2002).

Recent research indicates that there is considerable potential for biological control of *C. bergi*. Isolates of native Colombian strains of the entomopathogenic fungi *Metarhizium anisopliae* and *Paecilomyces* sp. have been evaluated in the lab. An *M. anisopliae* isolate parasitizing *C. bergi* in the field resulted in 61% mortality of fifth instar nymphs and an overall mortality of 33%. More recent studies with *M. anisopliae* strains CIAT 224 and CIAT 245 caused mortalities of 34.7% and 49.3%, respectively.

Applications of *M. anisopliae* (isolate CIAT 224), combined with a sublethal dose of the insecticide imidacloprid, were evaluated in the lab and greenhouse. *C. bergi* nymphal mortality was always significantly higher when *M. anisopliae* was applied in combination with imidacloprid, compared to applications of the fungus alone (80.3% vs. 34.2%). Thus, entomopathogens combined with sublethal doses of insecticides such as imidacloprid can be an effective tool in an IPM strategy for controlling *C. bergi* or other soil-borne pests; however, field studies are required before acceptable technologies can be recommended (Melo *et al*., 2006a).

Several species of nematodes have been identified parasitizing *C. bergi*. *Steinernema carpocapsae* successfully infected *C. bergi* in the lab, resulting in 59% parasitism after 10 days. Strains of *S. feltiae* and a native species of Colombia, *Heterorhabditis bacteriophora*, were compared in greenhouse studies with *C. bergi* adults. The penetration rate for *S. feltiae* was 93.9%, compared to 72.1% for *H. bacteriophora*; but *H. bacteriophora* caused higher mortality (42.2%) than *S. feltiae* (8.6%) after 15 days. Field studies are needed to evaluate the potential of *H. bacteriophora* and other nematode species in an IPM strategy (Melo *et al*., 2007).

12. White grubs

a. Taxonomy

A complex of rhizophagous white grubs (Scarabaeidae) is associated with the cassava crop in many regions of the Americas, Africa and Asia **(Table 1)**. White grubs are classified as hemi-edaphic (along with ants and termites) as they spend only a portion of their life cycle in the soil. It is during their larval stages in the soil that they can damage the cassava crop; the adult scarab beetles are not reported feeding on the above-ground parts of the plant. Recent surveys in cassava-growing regions of Colombia showed that white grubs were well represented in the edaphic communities associated with the crop. In Risaralda province 1,858 white grubs (eight species) were collected from cassava plots (Pardo-Locarno *et al*., 2005). It is often difficult to distinguish the species actually causing damage to the crop. The genus commonly associated with damage to cassava in the Neotropics and Africa is *Phyllophaga* spp. *Leucopholis rorida* is reported causing damage to cassava in Indonesia and other countries in Asia. Additional white grub species reported attacking cassava in Asia include *Lepidiota stigma*, *Aserica* sp. and *Holotrichia* sp. (Pardo-Locarno *et al*., 2005).

White grubs are generalist feeders, attacking many different hosts, and feeding opportunistically on cassava. It is often difficult to predict white grub attacks from one cropping cycle to the next, making white grub control more difficult. Adult grubs are winged beetles and highly mobile. Crops damaged by white grubs and reporting yield losses include: maize, pastures, potato, groundnut (peanut), grains, vegetables, sugarcane, beans, sweet potato, soybean and cassava.

b. Damage

White grubs damage cassava by feeding on stem cuttings being used to establish a new crop. Grubs feed on the bark and buds of recently planted cuttings, resulting in death and loss in germination. Feeding on secondary or feeder roots can result in rotting, plant dwarfism, wilting, poor plant growth and eventually death. Grubs can also attack swollen roots, causing fungal and bacterial infections and root rotting. There is an economic loss due to pesticide use and a reduction in crop value. Death of young plants reduces plant density,

resulting in increased weed problems. Grubs can cause plant death by feeding on the basal part of young stems.

In one field study in Colombia there was a 95% loss in stem cutting germination due to white grub attack. Cassava root yield losses of 25-30% due to *Phyllophaga* attack have been recorded in Colombia.

Recent studies in Colombia with *Phyllophaga menetriesi* with potted cassava plants under controlled conditions showed that one larva/plant caused a 30% reduction in plant survival, and three larvae/plant destroyed 50% of the plants in 56 days. White-grub feeding damage has also been observed on the roots including the swollen tuberous root. In field studies in Colombia, there was a relationship between the number of grubs present and root yield. One grub per plant resulted in about a 15% reduction, three grubs in a 40% reduction, five in a 80% reduction and seven and nine grubs in 100% yield loss (Ortega-Ojeda *et al*., 2007).

c. Biology and behavior

White grubs life cycle consists of the egg, three larval stages, the pupal and adult stages. Adults generally become active after the rains have started. The white eggs are oviposited singularly from one to several inches below the soil surface. Eggs hatch in two to four weeks and the young grubs feed on the roots and underground parts of the plants. The three larval stages and pupal stage will vary in length depending on the species. Laboratory studies with *P. menetriesi* resulted in an average of 13 days for the egg stage and 19, 27 and 175 days for the first, second and third instars, respectively. After the third instar, the larvae entered a diapause stage averaging about 30 days, followed by a pupal stage averaging 34 days. Adults remained in the pupal chamber for about 73 days, followed by a 15-day flight period. The complete egg-to-adult cycle of *P. menetriesi* averaged 386 days.

P. menetriesi is mostly observed at altitudes between 1000-1600 m, and damage to cassava is primarily during the rainy months when the crop is planted and early growth occurs (Pardo-Locarno *et al*., 2005).

The biology of *Leucopholis rorida* has been described on cassava in Indonesia. Adults become active after the rains have started and the most severe damage by the larvae occurs about 4 to 6 months later. The adult beetles initiate oviposition about 9 days after mating, laying up to 37 pearly white eggs singularly, 50 to 70 cm deep in the soil. Larvae hatch in about three weeks. The larval stage is about 10 months, with the 4 to 6 month-old larvae being the most destructive. Larvae live about 20-30 cm deep in the soil where they feed on cassava roots. Pupation occurs at a depth of about 50 cm. The prepupal stage is 14 days and the pupal stage is about 22 days.

d. Management

White grubs populations can often be detected during land preparation prior to planting. Farmer surveys in a major cassava-growing region in Colombia (Risaralda and Quindio provinces) disclosed that 71% of the farmers applied pesticides to control soil pests, while only 14% used biological control.

Numerous microbial agents for the biological control of white grubs have been identified **(Table 5)**. These include entomopathogenic nematodes, fungi, bacteria, viruses and protozoa. Fungal entomopathogens known to infect white grubs include: *Metarhizium anisopliae, Beauveria bassiana* and *Paecilomyces lilacinus*. Bacterial entomopathogens include *Bacillus popiliae* and *Servatia* sp. It should be noted that isolates or strains of these fungal or bacterial pathogens that have been isolated from insects or the soil may be pathogenic to specific white grub species. For example the CIAT collection contains 411 entomopathogenic fungal strains (*Metarhizium, Fusarium, Beauveria, Paecilomyces,* etc.), 89 bacterial strains (mainly from *Bacillus* spp.) and 15 entomopathogenic nematode strains (*Steinernema feltiae, S. krausseii* and *Heterorhabditis bacteriophora*). Strains or isolates of these microorganism will need to be evaluated against the white grub species causing damage to the cassava crop in a given area or region. In those areas where white grubs are a problem, an attempt should be made to collect, isolate and identify those strains of fungal or bacterial microorganisms that are parasitizing the white grub species present in cassava fields.

Biological control through the use of entomopathogenic nematodes and fungi offers promise for white grub control. A native Colombian strain of *Heterorhabditis bacteriophora*, when applied in high doses (10,000 infective juveniles/ml) to first and second instar larvae of *P. menetriesi* in lab studies, resulted in 88.3 and 83.4% mortality, respectively (Melo *et al*., 2007).

In lab studies at CIAT, several isolates of *M. anisopliae* caused high levels of mortality of *P. menetriesi*. Two isolates (CIAT 515 and CIAT 418) caused more than 60% white grub mortality. Isolate CIAT 515, in combination with a low rate of Imidacloprid, resulted in 90% mortality of second instar larvae.

In a greenhouse experiment the highest white grub mortality occurred when the fungus, *M. anisipliae*, was combined with the two insecticides. The second most effective treatment was the nematode, *Heterorhabiditis bacteriophora* together with the insecticide Fipronil. Observation with sub-doses of the insecticide potentially increased the effectiveness of the microorganism. This synergism is most effective when the insecticides and microorganisms are applied separately, indicating an additive effect between the two treatments (Melo *et al*., 2006b).

A greater than 70% larval mortality was achieved when the fungus was combined with the two insecticide treatments, whereas *M. anisopliae* alone resulted in about 15% mortality, similar to the control treatment. The nematode/Fipronil treatment resulted in a 47% mortality. Imidacloprid and fipronil alone gave a 42 and 32% mortality, respectively. The nematode treatment alone, resulted in only 2.5% mortality.

The greenhouse treatments that demonstrated the highest larval mortality were used in the field trials. In these trials no differences were observed between the three treatments employed (average of 44.6% mortality), but there was a significant difference when compared to the control. The third larval stage was used in field trials and this may account for the differences with the greenhouse trails where the second larval instar was used.

A diagnostic of root and cutting damage was carried out in the field trial. This may be a more valid indicator of treatment efficacy as it is difficult to determine the effect of microorganisms on the target pest in the field. The control treatment had the highest

percentage of dead cuttings while those treatments with Metarhizium and Imidacloprid had the lowest cutting death. In all treatments there was some cutting damage, averaging about 50%. This indicates that white grub damage will occur, regardless of treatment, but proper control can prevent high levels of cutting death.

It can be concluded that microorganisms such as fungi and nematodes can be applied with commercial insecticides at a reduced doses, and that this is a viable alternative for white grub control. White grub damage in the field will be significantly reduced and not cause high plant mortality. Generally, cassava plants can recover from this damage.

Strategies and technologies that should be implemented for white grub management:

Land preparation: Destroy and expose larvae and eggs to sun
Stake treatment: Imidacropid.
Crop rotation: cover crops, lemon grass (insecticidal properties)
Light traps
Application of lime to soil
Biological control: fungal pathogens, bacterial pathogens, entomopathogenic nematodes, predators
Resistant varieties: high HCN varieties reduce white grub populations
Insecticide: (last option) Imidacropid

13. Cassava root mealybugs

a. Taxonomy

Two mealybug species have been reported feeding on and causing damage to cassava roots. In South America, *Pseudococcus mandio* (Hemiptera: Pseudococcidae) has been recorded from southern Brazil, Paraguay and Bolivia; it is reported as causing root damage only in Brazil. *Stictococcus vayssierei* (Hemiptera: Stictococcidae) is reported from Cameroon and neighboring Central African countries. *S. vayssierei* is referred to in the literature as the root mealybug, the root scale or the brown root scale insect of cassava (Ngeve, 2003) **(Table 1)**.

b. Biology and behavior

P. mandio can result in reduced quality of tuberous roots and cause some plant defoliation. Females have three nymphal instars, and adults oviposit an average of 300 eggs, indicating a capacity for rapid population increases. The life cycle from oviposition to adult was found to be 25 days for females and 30 for males. Yield losses of 17% have been reported in southern Brazil (Pegoraro and Bellotti, 1994).

S. vayssierei larvae and adults attack young feeder roots of germinating stem cuttings, resulting in defoliation, wilting, tip dieback and plant death. Mature tuberous roots are often small, covered with mealybugs, and are unattractive for the commercial market. Females (males are rare) are dark red in color, circular and flattened. Eggs are protected by wax threads secreted beneath the female body. Larvae are creamy white and mobile.

c. Management

S. vayssierei infestations are more severe during the dry season and on unfertile, lateritic and clay soils. Infestations were more severe when cassava was planted on flat lands

than when planted on ridges. Plant vigor and root yield improved by approximately 22% when planted on ridges. Intercropping favored higher mealybug infestations than cassava grown in monoculture (Ngeve, 2003).

Adequate control measures have not been determined for either species. Recommendations for management of *S. vayssierei* in Cameroon include planting on ridges and monocropping cassava.

D. Secondary Pests

Numerous species of arthropods feed on cassava without causing major economic damage to the crop. These occasional or incidental pests may occur sporadically or at such low population levels that yield is not affected. If their populations increase or outbreaks occur in localized areas, some of these pests could cause yield losses. These secondary pests discussed briefly here include gall midges, termites, leafhoppers, leaf-cutting ants and grasshoppers.

14. Grasshoppers

a. Taxonomy

Zonocerus elegans and *Zonocerus variegatus* are potentially the most destructive of this group. They attack cassava primarily in Africa and are rarely reported feeding on cassava in the Neotropics (occasionally from Brazil). Several African countries, including Nigeria, Congo, Benin, Uganda, Ivory Coast, Ghana and Central Africa report thousands of hectares of cassava defoliated in some years, probably causing yield reductions.

b. Damage

Grasshoppers feed on the leaves, causing defoliation; but in heavy outbreaks, the young tender bark can be stripped. Young plants are preferred and attacks are more severe during the dry season. Yield losses as high as 60% have been estimated (Modder, 1994).

c. Biology and behavior

In Nigeria, grasshopper oviposition usually occurs at the onset of the rainy season; eggs hatch at the start of the dry season (6-7 months later). This population attacks cassava as the dry season progresses when other preferred herbaceous food plants become scarce. Experiments show that large amounts of HCN in the leaves can act as a deterrent to grasshopper feeding. The early instars (1-4) will not consume growing cassava, while instars 5 and 6 will eat it only if deprived of other food sources. Wilted cassava leaves are readily consumed by all stages and result in a high grasshopper growth rate (Bellotti and Riis, 1994).

d. Control

Chemical control of grasshoppers is feasible but may not be financially or ecologically sustainable, especially for small, resource-limited farmers. It is not considered an effective mid- or long-term solution as pesticide applications may lead to a resurgence of other pests such as the cassava mealybug or the cassava green mite when their natural enemies are killed indiscriminately (Modder, 1994).

Biological control with fungal entomopathogens offers a more effective long-term solution for grasshopper control. *Metarhizium flavoviride, Beauveria bassiana* and

Entomophaga grylli have been identified infecting *Z. variegatus*. Efforts are currently under way to develop effective biopesticides for grasshopper control. Results with *M. flavoviride* have been encouraging.

15. Gall midges

Iatrophobia brasiliensis (Diptera: Cecidomyiidae*)* has been recorded on cassava only in the Americas. They are considered of little economic importance and do not require control. However, the yellowish green or red galls on the upper leaf surface are highly visible to farmers, who may apply pesticides. A severe attack, especially on young plants, may cause leaf yellowing, and retarding of plant growth has been reported. Destruction of infested leaves is recommended to reduce midge populations (Bellotti, 2008).

16. Leaf-cutter ants

Several species of leaf-cutter ants (genera *Atta* and *Acromyrmex*) are reported feeding on cassava in the Neotropics, especially in Brazil **(Table 1)**. Commonly reported species are *Atta sexdens*, *Atta cephalotes* and *Acromyrmex landolti*. Ants cut semicircular pieces of leaves, which they carry to their underground nests. Cassava plants can be completely defoliated when a large number of worker ants attack a crop. Outbreaks occur most frequently during the early months of crop establishment, but plants usually recover from ant damage. Recent field trials in Venezuela resulted in a 55% reduction in root yield due to leaf-cutter ant defoliation. Ant nests are usually visible because of the mound of soil deposited around the hole. Control of leaf-cutter ants is difficult; toxic baits are recommended.

17. Termites

Termites are reported as pests in several cassava-growing regions of the world, but primarily in Africa **(Table 1)**. They attack cassava mainly in the tropical lowlands, feeding on stem cuttings, feeder roots, swollen roots or growing plants. In Colombia, termites have been observed causing losses in germination as well as death of young plants, especially in regions with sandy soils. Feeding on swollen roots can lead to root rot (due to soil pathogens) damage. Losses in germination of 30%, and 50% loss in stored planting material have been recorded. Control in the field is difficult, but stored planting material can be protected with an application of an insecticide dust (Bellotti *et al*., 1999).

18. Leafhoppers

Several species have been collected feeding on cassava **(Table 1)**. Several collections have been made by CIAT in Colombia, and numerous specimens from three families (Cicadellidae, Cixiidae and Delphacidae) are being identified. None is considered to be a pest causing yield losses, and all are usually observed in low populations. However, several of these species are being studied as possible vectors of cassava frog skin disease (CFSD), which probably originated in the Amazon regions of South America and has now spread to several countries in the region, causing considerable crop loss. The disease has been described as a virus of the family Reoviridae, and/or a phytoplasm. Damage is characterized by the suberization and thickening of the swollen root epidermis, resulting in low production of little commercial value. Several homopteran species have now been mass reared, and vector-transmission studies are being carried out (Calvert and Thresh, 2002).

FUTURE TRENDS AND CONSIDERATIONS

Climate Change and Crop Management

In recent years pest populations have shown dramatic increases on large-scale cassava plantations in southern Brazil. Crop damage resulting in root yield losses due to whiteflies, mealybugs and lacebugs have been recorded in the states of Sao Paulo, Parana and Mato Grosso do Sul. These increases in pest populations may be the consequence of climate changes in the region and subsequent changes in cassava crop management (Bale *et al*., 2002). Southern Brazil is sub-tropical and during the months of June, July and August, referred to as "winter", temperatures were often low enough to cause a frost, resulting in crop defoliation and stem damage. In June, cassava producers would prune plants back to almost ground level and store stems in protected confines, to be used as cuttings for planting in September when the threat of frost has past. The absence of cassava foliage in the fields probably caused pest populations to dramatically decrease.

With warmer temperatures in September, there was a regrowth of the pruned plants and, in addition, the next cropping cycle was sown, using the stored stems as a source of planting material (cuttings). Populations of mealybugs, whiteflies and lacebugs, did not increase to levels causing economic damage (yield losses). The major pest of consequence was the cassava hornworm (*E. ello*); this is a highly migratory species and adult populations probably moved into the southern region from warmer northern areas.

In recent years, according to cassava producers in this southern region, temperatures during this "winter" period have been warmer with less probability of frost. This has had an effect on cassava crop management practices. Farmers no longer prune all stems back to ground level, leaving growing stems and foliage in the field. This provides a food source for the afore-mentioned cassava pests. The life cycle is not disrupted and active pest populations can occur during this "winter" period. An active pest population is thus available in the field when regrowth occurs in September and when stem cuttings in the subsequent crop cycle germinate and young, tender plants emerge. Pest populations, especially whiteflies, can migrate to and infest the new growth and young plants. As earlier noted (see section on whiteflies) whitefly populations increase rapidly under these conditions and high populations can cause considerable yield losses.

In addition, the warmer temperatures and more frequent rainfall have resulted in more frequent, or staggered plantings of the crop. This has resulted in having the cassava crop at varying ages in the same field or plantation. These staggered plantings provide an ideal scenario for an increase in cassava pests, especially whiteflies.

Mealybug (*P. manihoti*) populations have also increased dramatically and this may also be due to the changes in management practices; especially if mealybug infested planting material is being used. Mealybug infested cassava stems, destined to be used as planting material (cuttings), have been observed on large plantations in the region.

Warmer temperatures may also be having an effect on the life cycle of the pest. It has been documented that the intrinsic rate of increase of mites, whiteflies, hornworms and mealybugs is more rapid as temperatures increase.

In the past, Asian cassava growing countries have been relatively free of arthropod pests causing yield losses, as none of the major cassava pests had been introduced into the region. This scenario may be the result of the distance and isolation of the region from cassava´s neotropical origin, combined with adequate quarantine measures. However, several minor or secondary arthropod species that can feed on cassava, but cause no yield reductions and little foliar damage in the Americas, have been inadvertently introduced into Asian cassava growing regions. These include the spiraling whitefly (*Aleurodicus disperses*), the mite, *Oligonychus biharensis*, and at least two mealybug species (*Ferrisia virgata* and *Pseudococcus jackbeardsleyi*. These species are of neotropical origin, have numerous alternate hosts, are infrequently found on cassava, and causing little on no damage to the crop in the Americas. They were most probably introduced into Asia on one of their alternate hosts. *A. dispersus, F. virgata* and *P. manihot*i, are presently causing considerable damage, including yield losses, to cassava in Thailand and possibly other countries in Asia. As noted earlier, *P. manihoti* is an important pest species that has caused yield losses in Africa and the Americas. The recent introduction of this species into Asia (Thailand) is of great concern and control strategies need to be implemented as soon as possible. The recent increased populations of these species on cassava in the region may be due to climatic changes, (e.g. warmer temperatures or altered rainfall patterns) combined with changes in crop management practices.

INTEGRATED PEST MANAGEMENT

The success of an ecologically oriented IPM program for cassava requires the implementation of a strategy that minimizes or prevents chemical pesticide use (Braun *et al*., 1993). Given the increased emphasis on commercial-scale plantations, where the crop has a high commercial value, there is a tendency to apply pesticides when noticeable crop damage occurs. Pests that trigger pesticide application include the cassava hornworm, whiteflies, lacebugs, mites, white grubs, burrower bugs, mealybugs and thrips.

Crop-protection technologies based on host plant resistance, microbial and arthropod biological control agents, together with appropriate agronomic practices, should be developed and implemented. This holistic approach has formed the basic philosophy for IPM research at international agricultural research centers such as CIAT and IITA, as well as in several national research programs such as EMBRAPA (Brazilian Agricultural Research Corp., Brasilia, Brazil), NARO (National Agricultural Research Organization, Uganda) (Bellotti *et al*., 1999).

CIAT, IITA and the Brazilian national program EMBRAPA maintain large germplasm banks that offer entomologists and breeders a potential pool for pest-resistance genes. Traditional farmers will adopt new varieties cautiously if they are adapted to local agro-ecological and socio-economic conditions. New or introduced varieties should not be highly susceptible to major pests in a given region. In the Neotropics, this is especially true for mites, whiteflies, thrips and mealybugs.

Biological control

Biological control involves the use of parasitoids, predators, pathogens or other antagonistic organisms, to suppress or maintain a pest population below economic damaging levels. Biological control may be employed against several different organisms, but it has

been most successful against arthropods (insects and mites). These include members of most of the important herbivorous orders: (Homoptera, Diptera, Hymenoptera, Coleoptera and Lepidoptera), but has been especially successful against the Homoptera (mealybugs, scale insects, whiteflies).

Natural enemies, the agents used in biological control, come from an array of taxonomic groups. Parasitoids, arthropods that kill their hosts, have been the most common type of natural enemy introduced for biological control of insects. Most parasitoids that have been employed in biological control strategies are in the orders Hymenoptera, and to a lesser degree Diptera. Predators consume their hosts and are very important in suppressing both native and immigrant herbivores, including insect and mite pests of agricultural crops such as cassava. Spider mites, such as the cassava green mite (*M. tanajoa*) have no parasitoid natural enemies, but numerous predator species can hold their populations in check. Pathogens, diseases of arthropod pests, include a range of bacteria, viruses, fungi and protozoa. They have been used successfully to control or suppress insect pest populations (Van Driesche and Bellows, 1996).

The principal biological control methods can be divided into three groups, conservation, the introduction of new natural enemy species, and augmentation. Conservation of those natural enemies that already exist in a given local is important in suppressing indigenous pests. The most important of the negative influences that can harm natural enemies are the indiscriminate use of chemical pesticides, especially those with broad spectrum action. Augmentation of natural enemies is employed when their populations are missing, late to arrive or too scarce or low to provide control. Natural enemy populations can be mass reared and released into fields in order to increase the native natural enemy populations. Augmentation may be directed against indigenous or exotic pests. The limitation to this method involves the costs, quality and field effectiveness of mass rearing and releases. Natural enemies can be expensive to rear and if the crop does not have a high cash value, the benefit may not be sufficient to warrant the additional cost.

In many areas, introduced or exotic species may comprise the major pests causing economic damage to the crop. In this case the introduction of new natural enemy species that are effective against the pest can be essential, and this approach has historically been very effective. The introduction of a key parasitoid to control the cassava mealybug (*P. manihoti*) in Africa is an excellent example of this method. The success of this method almost always involves the identification of the key natural enemy in the site of origin of the insect or mite pest. It appears that the major pests causing cassava crop losses in Asia are of exotic origin, and probably from the neotropics. The introduction of biological control agents from the Americas into Asia requires a high degree of scientific skill and adequate resources to insure a safe and effective solution to the problem. These classical biological control programs should be conducted by public or private qualified institutions with the appropriate expertise for successful results (Van Driesche and Bellows, 1996).

Biological control agents have been identified for many of the cassava arthropod pests **(Table 2-5)**. The efficacy of naturally occurring biocontrol agents to maintain pests below economic damage levels has been well documented in certain cases but considerable research still needs to be accomplished. Classical biological control has been successful in Africa against two introduced pests from the Americas: the cassava mealybug (*P. manihoti*)

and the green mite (*M. tanajoa*). Although natural biological control is probably effective controlling some pests in the Neotropics, pest outbreaks and subsequent yield losses continue to occur. For example, the hornworm *Erinnyis ello* has a large complex of natural enemies including predators, parasites and pathogens; however, they are not effective in maintaining the hornworm below the economic injury level. The adult's migratory abilities and sporadic attacks serve as a defense against the more than 30 natural enemies. The stemborer *Chilomima clarkei* causes considerable damage in certain regions of Colombia, but effective natural enemies have not been identified. In recent years whitefly populations and damage have increased in several regions of the Neotropics as well as in Africa and Asia, causing considerable yield reduction. Several natural enemies have been identified, but their role in a biological control program has not been determined.

It should be kept in mind that in cropping systems where cassava is grown as a functional perennial, certain pests and their associated natural enemies may be in equilibrium. When cassava is grown year round in the tropics, often with overlapping cycles, pest species may be present throughout the crop cycle and thereby able to increase rapidly when environmental conditions become favorable to their dynamics. Natural enemy populations may not respond rapidly enough to suppress the increasing pest populations so outbreaks occur. Populations of mites, mealybugs, lacebugs and whiteflies, although present in the subtropics of the Americas, do not increase as rapidly or reach the levels of their counterparts in the tropical regions. During the 'winter' months in subtropical regions, cassava will lose most or all its foliage. This can cause considerable reduction in pest populations so any increases may be retarded when warmer, more favorable, growing conditions return in the spring.

Host Plant Resistance

Biotechnology tools offer the potential for developing improved pest-resistant cultivars and enhancing the effectiveness of natural control organisms including parasitoids and entomopathogens. Wild *Manihot* species are a rich source of useful genes for the cultivated species *M. esculenta* and for resistance to pests and diseases. Their use in regular breeding programs is restricted by the long reproductive breeding cycle of cassava and 'linkage drag' associated with the use of wild relatives in crop improvement. This source of resistance genes has been exploited for controlling CMD in Africa. CMD resistance was obtained by intercrossing cassava varieties with *Manihot glaziovii*, which resulted in interspecific hybrids that were backcrossed to cassava until CMD-resistant varieties were produced.

Several wild *Manihot* species have been evaluated in the greenhouse and field for resistance to mites (*M. tanajoa*), mealybugs *(P. herreni*) and whiteflies *(Aleurotrachelus socialis*). Genotypes (accessions) of the wild species *Manihot flabellifolia* and *Manihot peruviana* displayed intermediate levels of resistance to *M. tanajoa* and *P. herreni* and high levels of resistance to *A. socialis*. In addition, *M. tanajoa* oviposition was greatly reduced when feeding on accessions of *Manihot alutacea* and *Manihot tristis*. Interspecific crosses between these wild *Manihot* species and *M. esculenta* landrace varieties have resulted in numerous interspecific progeny, which are being evaluated for pest resistance. Initial results indicate that the resistance is heritable as numerous progeny have been identified with resistance to *M. tanajoa* and *A. socialis*. Three polymorphic molecular markers for *M. tanajoa* that showed clear differences between resistant and susceptible individuals were

identified in *M. flabellifolia*. A project is under way to develop low-cost tools for accelerated marker-aided introgression of useful pest-resistance genes into cassava gene pools.

CONCLUSIONS

It is predicted that cassava production in Africa, Asia and the Americas will increase considerably during the next decade. This growth will be market driven and influenced by the processing and private sectors. Cassava can provide the raw material for the animal feed, starch and bio-fuel industries, as well as remaining an important food for human consumption. Pest management will continue to play an important role in sustaining high cassava-production levels. This will require continued research inputs to develop new integrated pest management (IPM) technologies.

In order to meet the demand for increased cassava production, farmers will seek new higher yielding varieties. This will increase the movement of germplasm – usually vegetative stem cuttings – between regions, countries and even continents. Quarantine measures to prevent the movement of pests, especially into Asia, are an important issue. Cassava pests have shown the ability to disseminate great distances as evidenced by the introduction of the mite and mealybug into Africa from the Americas. There are several additional pests that could cause severe crop losses if introduced into Africa or Asia, including several mite species, lacebugs, whiteflies, stemborers, mealybugs and thrips (**Table 1**). Moreover, what may be considered a secondary pest in the Neotropics could become a major pest outside its center of origin, as evidenced by the mealybug, *P. manihoti*. Cassava researchers, especially entomologists need to be pro-active in meeting the demands of increased cassava production and an evolving environment.

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CHAPTER 11

CASSAVA DISEASES IN LATIN AMERICA, AFRICA AND ASIA [1]

Elizabeth Alvarez[2], Germán Alberto Llano[2], and Juan Fernando Mejía [2]

ABSTRACT

The importance of cassava (*Manihot esculenta* Crantz) in tropical regions of the world is growing because of an increase in both consumption of fresh cassava roots and the numerous agro-industrial uses of the crop. World cassava production totaled 224 million tonnes in 2007, with the greatest production in Africa. Like all other crops, cassava is also infected by several pathogens. Among the various diseases of cassava that limit production, Cassava Bacterial Blight (CBB), Cassava Mosaic Disease (CMD), Cassava Root Rot (CRR), Cassava Mosaic Virus (CMV), Cassava Brown Streak Disease (CBSD) and Cassava Frogskin Disease (CFSD) are the most important.

In **South America (**Colombia, Brazil and Venezuela), Cassava Bacterial Blight (CBB), caused by *Xanthomonas axonopodis* pv. *manihotis* is considered as perhaps the most limiting disease in cassava as it can cause a total loss of the crop. The bacterium disseminates widely through stakes from infected plants, it disperses through splashing during rainfall and by contaminated tools. Super-elongation disease, caused by *Sphaceloma manihoticola* is one of the major diseases affecting cassava in Colombia, Brazil, Venezuela and Central America. Among susceptible cultivars, this fungal disease can cause yield losses of up to 80%. The disease disseminates through infected stakes. Frogskin disease (CFSD) is considered of economic importance as it affects directly root yields, causing losses of more than 90% in some parts of Colombia. The disease is associated with a phytoplasma and has also been reported in Brazil, Costa Rica, Panamá, Perú and Venezuela. The disease has not been reported in Asia and Africa. Improved quarantine inspection is needed to prevent its introduction.

Cassava Root Rot, induced by *Phythophthora* species, can cause losses of up to 80% of total production in Colombia. The pathogen has been identified as *Phytophthora palmivora* and *Phytophthora tropicalis*. In Brazil *Phytophthora drechsleri* can cause significant damage in crops, and losses can reach 80 to 100% when susceptible cultivars are grown

In **South and Central America**, Cassava Common Mosaic Virus (CsCMV), a potexvirus, and Cassava Vein Mosaic Virus (CVMV) (Caulimoviridae) have been described. CsCMV was first reported in Southern Brazil and Paraguay; it has no known vector and its spread in the field is attributed to mechanical transmission. CVMV is common in the semi-arid zone of Northeastern Brazil.

In **Africa**, Cassava Common Mosaic Virus (CsCMV) has been detected only once in material assumed to have been introduced from South America where the virus is prevalent.

Cassava Mosaic Disease (CMD) is caused by several cassava mosaic geminiviruses and is recognized as the most important constraint to the production of cassava in Africa. A total of eight

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[1] This is an updated version of the paper entiteled "Cassava Diseases" which has been translated from the original Spanish, entitled *Enfermedades del Cultivo de la Yuca y Métodos de Control* by Elizabeth Alvarez and Germán Llano, Chapter 8 of *La Yuca en el Tercer Milenio: Sistemas Modernos de Producción, Procesamiento, Utilización y Comercialización*, published by CLAYUCA, Cali, Colombia, 2006. Also available at http://www.clayuca.org/public_libro.htm.

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distinct cassava-infecting geminivirus species have now been identified. Synergism between two species of geminiviruses, the African Cassava Mosaic Virus (ACMV) and the East African Cassava Mosaic Virus (EACMV) is an important factor for initiating and promoting an epidemic. New types of geminivirus satellites in cassava from East Africa, which are associated with severe CMD, have been discovered. These disease complexes pose a serious threat to tropical and subtropical agro-ecosystems worldwide.

Cassava Brown Streak Disease (CBSD) is considered important in Tanzania, Uganda, Mozambique and coastal Kenia; there is still uncertainty concerning its epidemiology and mode of spread.

Cassava bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *manihotis (Xam),* is a destructive disease in Africa and affects both yields and planting material leading to yield reductions. CBB was particularly severe in all ecozones of Benin, but is rare in Ghana. Cassava in the savanna zones of Benin was heavily diseased with an incidence of 34-84% and a high percentage of plants showed systemic symptoms, while CBB was not observed in the rainforest zone and only sporadically in the coastal savanna zone of Ghana. High incidence and severity of CBB was associated with increasing plant age and clay soils, whereas the disease was less severe when fields were intercropped or planted to cultivar mixtures

In **Asia c**assava is a commercial crop entering diversified markets.Where soils are marginal in fertility, and rainfall is uncertain, cassava has a strong adaptive advantage. Among the various diseases of cassava that limit production, CMD (in India only) and CRR are of major importance as they cause a considerable reduction in yield. CMD is widespread in almost all cassava growing areas of India and causes yield losses of up to 80% in susceptible varieties, and up to 50% in field tolerant varieties. CMD is caused by a cassava mosaic begomovirus. Another important disease is root rot caused by *Phytophthora palmivora,* which is emerging as a serious threat to cassava in several industrial areas of Tamil Nadu State of India, causing up to 50% loss in endemic areas. It is also severely affecting yields in Bangladesh and is observed localized in wet areas throughout Asia. Heavy soils, excessive irrigation, poor drainage and the development of a hard pan favor the disease. Disease incidence depends on climatic factors, nutrient management and genotypes.

Cassava diseases such as super-elongation, vein mosaic and brown streak have not been reported in India or elsewhere in Asia. Plant quarantine measures need to be strictly implemented to avoid their introduction

For all these diseases, effective management strategies and healthy planting material production, are discussed, with emphasis on those diseases currently prevalent or being a major future threat in Asia.

INTRODUCTION

World production of cassava roots was estimated at 233 million tonnes in 2008. Africa was the largest producer with 118 million t on almost 12 million ha, followed by Asia with 78.7 million t on 3.97 million ha. Cassava (*Manihot esculenta* Crantz) is a significant staple, providing a basic daily source of dietary energy for almost one billion people in 105 countries. It also has numerous agro-industrial uses. Cassava grows on marginal lands, tolerates drought, and can grow in low-fertility soils. Cassava is also the most inexpensive source of starch that exists, being used in more than 300 industrial products (FAOSTAT, 2010).

Cassava is still widely cultivated under traditional management. This suggests that large numbers of farmers may be ignorant of the crop’s diseases and their integrated

management. Hence, several diseases threaten the sustainability of cassava production and its profitability. The principal diseases attacking the crop are:

Cassava bacterial blight (CBB; *Xanthomonas axonopodis* pv. *manihotis* or *Xam*)
Phytophthora root rots (PRR; *Phytophthora* spp.)
Superelongation disease (SED; *Sphaceloma manihoticola*)
Cassava frogskin disease (CFSD; phytoplasma Cfdp of the 16SrIII group)
Cassava mosaic disease (CMD; begomovirus complex)
Cassava brown streak disease (CBSD; an ipomovirus).
Brown leaf spot (*Cercosporidium henningsii)*
Diffuse leaf spot (*Cercospora vicosae*)
White leaf spot (*Phaeoramularia manihotis*)
Anthracnose (*Colletotrichum spp.*)

Of these, the most common diseases found in Asia, are Cassava Bacterial Blight (CBB), Phytophthora root roots, Indian and Sri Lanka Cassava Mosaic Disease (only in India and Sri Lanka), Anthracnose and Cercospora. Recently, a new, as yet unidentified disease with symptoms of Witches Broom has been observed in Vietnam and Thailand.

DISEASES CAUSED BY FUNGI

1. Superelongation Disease (*Elsinoe brasiliensis*)

Importance

Superelongation disease (SED) attacks susceptible cultivars, especially during the rainy seasons. Damage caused by SED is highly variable, depending on the level of cultivar resistance, climatic conditions, concentration of the initial inoculum, and the degree of contamination of planting materials (Alvarez and Llano, 2002).

Losses can exceed 80% of total production in young crops, whereas significant losses do not occur in crops that are more than six months old. In Colombia, SED is found in the Eastern Plains, Atlantic Coast, and the inter-Andean valleys. The disease is acute in agro-ecological areas with annual mean temperatures of 28ºC and annual precipitation of more than 1500 mm. In the greenhouse, 8 hours of misting at temperatures of 25º to 30ºC was sufficient to cause an outbreak, indicating how easily the pathogen develops in the field (Mejía, 2001).

Distribution

Superelongation disease was first observed by Bitancour and Jenkins in 1950, on *Manihot glaziovii* Muell.-Arg. in Brazil and Nicaragua and on *M. esculenta* in the Dominican Republic and Guatemala. The disease has since been reported (in order of reporting year) in Costa Rica (Larios and Moreno, 1976), Colombia (Lozano and Booth, 1979), Mexico (Rodríguez, 1979), Cuba (Pino, 1980), Venezuela (Rondón and Aponte, 1981), the Dominican Republic (Sosa, 1992), Barbados, Panama (Chávez, 1992; Zeigler, 2000), Brazil (where it is restricted to the western regions of the country) (Alvarez *et al*., 2003b), and Trinidad and Tobago (Reeder *et al*., 2008). At the end of 2008, the disease was detected in Thailand (Elizabeth Alvarez, 2008, personal communication). The disease appears to be unknown in Africa.

Symptoms and epidemiology

The characteristic symptom of this disease is the exaggerated lengthening of stem internodes (Zeigler *et al*., 1980), creating thin and weak stems. Diseased plants are much taller and/or weaker and spindlier than healthy ones. In green sections of stems, and in petioles and leaves, deformations develop in associations with cankers. The lens-shaped cankers often have dark margins and are variable in size. In leaves, cankers are found on the underside, along the primary or secondary veins. In stems, they may be more diffuse. Frequently, young leaves curl, and do not develop fully nor do the leaf blades expand completely. Leaves also develop irregular white spots (**Figure 1**[1]). Sometimes partial or total death of leaves occurs, resulting in considerable defoliation. Dieback of the plant may also occur.

The disease spreads from one place to another through the use of infected stakes. The principal focus of infection frequently constitute the shoots originating from residues of old plants left in the field after harvest. The disease spreads rapidly during the rainy season. This rapid dissemination is believed to occur through the formation of spores in the cankers. These spores can survive for more than six months in infected plants and are carried by rain and wind.

Etiology

Superelongation disease is caused by the fungus *Elsinoe brasiliensis*, which initially grows on the epidermis of the host and, after penetration, grows in the intercellular spaces in tissues of the epidermis and cortex. The fungus produces gibberellins, which promote the exaggerated growth in the plant's internodes. Gibberellins, as suggested by previous studies for other pathogens (Muromtsev and Globus, 1975), play an essential role in the fungus's nutrition. The fungus, which has a low production of hydrolytic enzymes, uses this hormone to obtain sugars from the plant, promoting, at the molecular level, hydrolysis of carbohydrates with greater mass (Mejía, 2001).

According to Alvarez and Molina (2000), the pathogen's genetic diversity in Colombia is broad, presenting differences among isolates within a single location and between locations. Isolates from the Atlantic Coast, Eastern Plains, and inter-Andean valleys of Colombia, and from central and southern Brazil, comprise two evolutionary units, with each unit relating to its respective country (Alvarez *et al*., 2001).

For gene 18S rRNA, obtained from two isolates of *E. brasiliensis*, the sequencing of a region involving ITS1 and ITS2 was reported to GenBank (accessions AY739018 and AY739019; CIAT 2004).

Host range

Elsinoe brasiliensis and *Sphaceloma* species (the asexual state), which both attack cassava, have a wide range of Euphorbiaceae hosts, including *Euphorbia brasiliensis* L., *E. hypericifolia* L., *Jatropha aconitifolia* Muell. var. *papaya* Arbelaez, *J. curcas* L., *Manihot carthaginensis* Muell., *M. esculenta*, and *M. glaziovii*. These hosts are cosmopolitan weeds and widely cultivated ornamentals.

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[1] For color photos see pages 750-760.

Many regions in Africa and Asia have climatic conditions that closely resemble to those of the Eastern Plains, Atlantic Coast, and inter-Andean valleys of Colombia, where the pathogen causes considerable losses. These African and Asian regions therefore face the danger that the pathogen will be introduced through planting materials of ornamentals such as *Jatropha* spp. L., which are not necessarily restricted by the same sanitary regulations as cassava.

Because the host range is broad, completely eradicating the pathogen is impossible and a certain amount of sufficient inoculum will be present throughout the year. In Brazil, the weed *Euphorbia heterophylla* L. was shown to be host to strains of *Elsinoe brasiliensis* that were highly pathogenic to cassava (Alvarez *et al*., 2003b). Furthermore, the genetically very variable hosts are also able to maintain a variable population of the pathogen (Zeigler, 2000).

Integrated disease management

The use of healthy planting materia, obtained from disease-free plants or from plants derived from meristem culture, comprises a tool that may be sufficient to maintain disease-free crops. However, one preventive method for eradicating the pathogen is to immerse infected stakes for 10 min in Captafol at 4.8 g/l of active ingredient (a.i.). When symptoms are observed in the field, foliar spraying should be carried out with Difenoconazole at 2.5 cc/l, followed by crop rotation with grasses.

In areas where the pathogen is endemic, planting should be carried out during periods with the least precipitation (CIAT, 2003a). Infected plants (cassava or other Euphorbiaceae hosts) should be destroyed as soon as they are identified. The best way to eliminate this material is to pull up infected plants and burn them *in situ* (Zeigler, 2000).

Varietal resistance

The selection of resistant varieties is perhaps the best alternative for controlling SED. Between 1995 and 2007, CIAT evaluated about 6400 genotypes at Villavicencio (Colombia) and found 257 with resistance to SED. On-farm evaluations at Sincelejo (Sucre, Colombia) showed the following as resistant: MVen 25 and CM 4843-1, followed by ICA Catumare, ICA Cebucán, ICA Negrita, Vergara (CM 6438-14), and CM 4574-7 (CIAT, 2001; 2002b; 2003b).

Pathogenic races of *E. brasiliensis* exist and are of high genetic variability. While they should be taken into account when improving resistance to SED (Alvarez and Molina, 2000; Alvarez *et al*. 2003b), they are not thought to pose serious constraints to varietal improvement (Zeigler, 2000).

Biological control

Spraying with suspensions of *Pseudomonas putida* considerably reduced the severity of damage caused by SED, thereby significantly increasing cassava yields (CIAT, 1985).

2. Brown Leaf Spot (*Cercospora henningsii*)

Importance

Brown leaf spot has a broad geographical distribution, being found in Asia, North America, Africa, and Latin America. It attacks naturally *M. esculenta*, *M. glaziovii*, and *M. piauhynsis* Ule (Ferdinando *et al*., 1968; Golato and Meossi, 1966; Powell, 1972). In India, *Cercospora henningsii* is an important pathogen, causing severe defoliation (Edison, 2002).

Symptoms and epidemiology

Symptoms in cassava leaves are characterized by leaf spots visible on both sides. On the leaves' upper surface, uniform brown spots appear, with defined and dark margins. On the leaves' undersurface, the lesions have less-defined margins, and, towards the center, the brown spots have a gray-olive background because of the presence of the fungus' conidiophores and conidia. As these circular lesions grow, from 3 to 12 mm in diameter, they take up an irregular angular form, their expansion being limited by the leaves' major veins (**Figure 2**).

The veins found within the necrotic area are black. Sometimes, depending on how susceptible the variety is, an undefined yellow halo or discolored area can be observed around the lesions. As the disease progresses, infected leaves become yellow and dry before falling off, possibly because of toxic substances secreted by the pathogen. Susceptible varieties may undergo severe, or even total, defoliation during the hot rainy season.

When wind or rain carry conidia that have dropped from wounds of infected tissues towards leaves of a new planting, primary infections occur. If the humidity is sufficiently high, the conidia will germinate, producing branched germinal tubes that frequently anastomose (Chevaugeon, 1956; Viégas, 1941).

When lesions mature, stromata appear from which conidiophores emerge. Secondary cycles of the disease are repeated throughout the rainy season, when wind or rain carries conidia to new susceptible tissues of the plant. The fungus survives the dry season in old lesions, frequently those of fallen leaves. It renews activity with the advent of the rainy season and the growth of new leaves of the host.

Chevaugeon (1956) observed that in a cassava plant the lower leaves are more susceptible than the youngest leaves. However, certain susceptible species (e.g., *M. carthagenensis* Muell.) and *M. esculenta* cultivars can be severely attacked. Severe symptoms have been observed in young leaves, petioles, and even fruits of *M. carthagenensis*. Although plants "hardened" by unfavorable conditions appear more resistant, no significant differences in susceptibility were found between plants growing in fertile soils and those growing in poor soils (Chevaugeon, 1956).

Etiology

Cercospora henningsii, causal agent of the disease, grows in the intercellular spaces of leaf tissues, producing stromata from which conidiophores are produced in dense fascicles. The conidiophores are pale olive brown, semi-transparent, with uniform width and color, and non-branching. Sometimes, black perithecia appear, disseminated in the

necrotic tissue of leaf spots and on the leaves' upper surface (Powell, 1972). The perfect state of *C. henningsii* is *Mycosphaerella manihotis* (Ghesquière, 1932; Chevaugeon, 1956).

Management and control

To reduce the severity of infection, recommended cultural practices include reducing excess humidity during planting (Golato and Meossi, 1966). Fungicides based on copper oxide and copper oxychloride, suspended in mineral oil, and applied at 12 l/ha also provide good chemical control (Golato and Meossi, 1966). The best control over the disease can be achieved by using resistant varieties. Significant differences in varietal resistance have been found in Africa (Chevaugeon, 1956; Umanah, 1970), Brazil (Viégas, 1941), and in the extensive collection of cassava varieties held at CIAT, Colombia (CIAT, 1972).

3. Diffuse Leaf Spot (*Cercospora vicosae*)

Importance

This disease is found where brown leaf spot predominates, that is, in the hot cassava-growing areas of Brazil and Colombia (CIAT, 1972; Viégas, 1941). The pathogen causes severe defoliation in susceptible cultivars, but in Colombia does not cause serious crop losses.

Symptoms and epidemiology

This disease is characterized by the presence of large leaf spots, with undefined margins. Each spot may cover one fifth, or more, of the leaf lobe. On the leaves' upper surfaces, the spots are uniformly brown, whereas, on the lower surfaces, spots also have grayish centers caused by the presence of the fungus' conidia and conidiophores. The spots' general appearance is similar to that of the spots induced by *Phoma* sp., although lesions induced by the latter have concentric rings on the leaves' upper surfaces (**Figure 3**).

Defoliation may occur in susceptible cultivars, being more severe at the end of the rainy season and/or vegetative cycle. As the disease progresses, leaves become yellow and dry before falling off.

Symptoms of this disease can be confused with those of cassava bacterial blight (CBB; see below), except that the blight lesions are noticeably aqueous.

Etiology

The fungus does not form stromata but sporulates abundantly. The conidiophores are reddish dark brown (Chupp, 1953). The fungus has been recorded as a pathogen occurring only on *Manihot* spp. Mill. As its incidence on a single plant or in a given planting is very low and apparently confined to the plant's lower leaves, its importance is relatively less.

Management and control

- Planting with healthy and resistant cultivars
- Using cultural practices that reduce humidity during planting

4. White Leaf Spot (*Phaeoramularia manihotis*)

Importance

This fungus is commonly found in the cold humid cassava-growing regions of Asia, America, North America, tropical Africa, and Latin America (Castaño, 1969; Chevaugeon, 1956; CIAT, 1972). In these areas, the pathogen may cause considerable defoliation in susceptible varieties of *M. esculenta*, the only known host species (Chevaugeon, 1956; Viégas, 1941).

Symptoms and epidemiology

Leaf spots caused by *P. manihotis* are smaller, with a different color, to those induced by *C. henningsii*. They vary from circular to angular, with diameters of usually 1 to 7 mm. They are normally white, but sometimes yellowish brown. Lesions are sunken on both sides, to half of the thickness of a healthy leaf blade. On the lower leaf surface, the white spots can be distinguished but they frequently have diffusely colored margins, which sometimes appear as brown-violet irregular lines, surrounded by brown or yellowish halos. The spots' centers have a velvety grayish aspect during the pathogen's fruiting (**Figure 4**).

The fungus penetrates the host through stomatal cavities and then invades the host's tissues through the intercellular spaces. When leaf spots reach 5 to 7 mm in diameter, a stroma is formed, which produces conidiophores. The disease's secondary cycles are repeated throughout the rainy season as conidia are dispersed by wind or rain splash. The fungus survives the dry season in old infected tissues and renews activity at the beginning of the rainy season and with the host's new growth.

Etiology

Phaeoramularia manihotis, the causal agent, forms thin stromata in lesions on leaves. The stromata produce conidiophores in loose fascicles that emerge through the stromata and are usually olive brown (Powell, 1972).

White leaf spot is very similar to brown leaf spot. However, brown spot usually occurs in warm but not humid areas, whereas white spot appears in cold humid areas. These differences in their geographical distribution are also observed in Africa and Latin America, and are probably the result of different responses of the respective causal agents to temperatures and humidity. The optimal temperature for germinating *C. henningsii* conidia is 39ºC, with a maximum temperature of 43ºC. For *P. manihotis*, these temperatures are, respectively, 33ºC and 43ºC (Chevaugeon, 1956).

Management and control

The control measures recommended for this disease are similar to those for brown leaf spot. Specifically resistant varieties are unknown, but field studies suggest they exist (J. Carlos Lozano, 1979, unpublished data).

5. Concentric Ring Leaf Spot (*Phoma* spp.)

Importance

This fungal disease, caused by *Phoma* spp., usually appears in the cold cassava-growing areas of Colombia (CIAT, 1972), Brazil (Viégas, 1943a), Philippines, tropical Africa, and India (Ferdinando *et al*., 1968). According to Edison (2002), this disease is an

emerging problem in India in certain areas where cassava cultivation is intensive. During the rainy season and when the temperature is below 22ºC, the disease may cause severe defoliation in susceptible varieties and almost always produces stem dieback.

Symptoms and epidemiology

The disease is characterized by the presence of large dark brown leaf spots, with usually undefined margins. These lesions are commonly found at leaf points, margins of leaf lobes, or along the central vein or other secondary veins. Initially, lesions appear as concentric rings of brown pycnidia on the leaf's upper surface (**Figure 5**). These rings are not found on old injuries because the rain drags away mature pycnidia. In these cases, the spots are uniformly brown, and are very similar to those caused by *Cercospora vicosae*. On the lower leaf surfaces, very few pycnidia occur. Hence, lesions are uniformly brown.

Under conditions of high relative humidity, lesions may be covered by braid-like chains of grayish-brown hyphae. On the lower leaf surfaces, the nervures within the lesions become necrotic, forming black bands that emerge from the spots. These spots grow, causing leaf blight. The fungus invades the infected leaf and then the petiole, which becomes dark brown as it necroses. Leaves wilt and then fall, resulting in severe defoliation in susceptible cultivars. These cultivars may present dieback during epiphytotes and even total plant death. Necrotic stems become dark brown and frequently appear covered with pycnidia.

Field studies suggest that the more mature lower leaves may be more resistant than the young upper leaves. However, total defoliation, accompanied by partial or total dieback, has been observed in susceptible cultivars.

Favorable conditions for the germination of fungal spores occur at temperatures between 20º and 25ºC. With artificial inoculation, infection is only achieved when inoculated plants are kept for 48 h at less than 24ºC and with 100% relative humidity (J. Carlos Lozano, 1979, unpublished data). Under field conditions, this disease always occurs during the rainy season and in areas where the temperature is less than 22ºC.

The fungus' survival mechanism during dry hot periods is unknown. Viégas (1943b) suggested that the fungus may produce its sexual state on infected stems and leaf residues. However, this has not yet been observed or recorded.

Etiology

The causal agent produces numerous, spherical, dark brown pycnidia, either individually or in small clusters, on surfaces of leaves or stems. Pycnidia measure 100 to 170 µm in diameter, their walls are formed by polyhedral cells; and their ostiole measures 15-20 µm. Conidiophores are short and hyaline, producing small conidia (15-20 µm) that are unicellular and ovoid or elongated (Ferdinando *et al*., 1968; Viégas, 1943a). On lima-bean agar, the fungus forms pycnidia in profuse quantities, appearing in concentric rings.

Management and control

To date, no measures of control exist for the disease, even though it causes heavy losses in areas where environmental conditions are propitious for its development. Although no reports exist on varietal resistance, in the field in Colombia, resistance has been observed in naturally infected plantings. Chemical treatments such as carbendazim (3 g a.i./l) and

benomyl (0.6 g a.i./l) during the rainy season may be equally effective in those areas where the disease is endemic.

6. Cassava Ash (*Oidium manihotis*)

Importance

This disease was first recorded in Africa in 1913 (Saccardo, 1913) and has since appeared in Latin America (CIAT, 1972; Viégas, 1943a) and Asia (Park, 1934). The disease is characterized by the presence of yellowish undefined spots on *M. esculenta* leaves. Although it is widely disseminated and frequently occurs during the dry season, the disease is considered to be of minor importance as it usually attacks only the lower leaves, in which it induces some necrosis.

Symptoms and epidemiology

The first symptoms of the disease are characterized by the appearance of a white mycelium that grows on the leaf surface (**Figure 6**). The fungus penetrates the host cells, using haustoria. The infected cells become chlorotic and form undefined yellowish lesions. Within these yellowish areas, pale brown necrotic areas frequently appear. These are angular in shape and of different sizes. In some cassava varieties, the disease stops in the state of yellowish undefined lesions, which then may become confused with those induced by insects and mites.

Fully developed mature leaves seem to be most susceptible to pathogenic attack, although the young leaves of some varieties may also present symptoms. The disease commonly appears during the dry season and in warm areas.

Etiology

The sexual state of the causal agent, *Oidium manihotis*, is *Erysiphe manihotis* (Ferdinando *et al*., 1968). The fungus' mycelium is white, producing numerous haustoria on the host's epidermis. Conidiophores rest in an erect position. They are simple, with the upper parts both longer and wider, as they form the conidia. Conidia are oval or cylindrical, unicellular, hyaline, and measure 12-20 × 20-40 µm. They are produced in basipetal chains (Ferdinando *et al*., 1968; Saccardo, 1913; Viégas, 1943b).

Management and control

Although specific control of the disease is considered unnecessary, observations suggest that resistant varieties exist (CIAT, 1972). Ferdinando *et al*. (1968) suggest that spraying with sulfur-based compounds can control the disease.

7. Cassava Anthracnose (*Glomerella manihotis*)

Although cassava anthracnose has been known for a long time, it has been considered of minor importance. It is characterized by the presence of sunken leaf spots, 10 mm in diameter, that are similar to those caused by *C. henningsii*. The latter, however, appear towards the base of leaves, thus causing their total death.

The pathogen also causes young stems to wilt and induces cankers on mature stems (Irvine, 1969) (**Figure 7**). New leaves, produced at the beginning of the rainy season, are

the most susceptible. The disease tends to disappear when the dry season begins (Irvine, 1969). This finding agrees with results obtained from artificial inoculations with an aqueous suspension of spores from the pathogen. Inoculation is successful if incubation is at 100% relative humidity for 60 hours. The fungus will stop invading plant tissue when the relative humidity drops to 70% (CIAT, 1972). The insect *Pseudotheraptus devastans* Distant is associated with the disease (Fokunang *et al*., 2000), contributing to the pathogen's dissemination and increasing the severity of symptoms.

The organism causing this disease has been variously called *Glomerella manihotis*, *Colletotrichum manihotis* (Vanderweyen, 1962), *Gloeosporium manihotis* (Bouriquet, 1946), and *Glomerella cingulata* (Irvine, 1969). All these names possibly refer to one species, but this hypothesis is yet to be confirmed.

Stem anthracnose caused by a *Colletotrichum* sp. was recorded in Nigeria (IITA, 1972). Green portions of the stems presented shallow oval depressions that were pale brown, but with a point of normal green tissue in the center. In the ligneous portions of the stems, lesions were round, swollen, and in bands, forming deep cankers on the epidermis and cortex, and sometimes deforming the stem. Its importance is unknown but its prevalence, occurrence, and dissemination are considerable. In Asia stem anthracnose was observed in Thailand (Alvarez, 2009, personal communication).

8. Cassava Rust (*Uromyces* spp.)

Importance

Although recorded in Brazil and Colombia, this disease is considered to be of minor importance. It appears at the end of dry periods, sometimes causing a type of shoot proliferation in stem apices (Normanha, 1970).

Symptoms and epidemiology

Infection is characterized by pustule formation on leaf veins, petioles, or green branches (**Figure 8**). Pustules are light to dark brown, depending on their age or class of fungal fructification. Mature pustules are readily parasitized by the fungus *Darluca filum*. They are sometimes surrounded by chlorotic halos, and, usually, induce deformation of affected parts. Wind is the principal dissemination agent.

Etiology

In cassava, several species of rust pathogens have been recorded in different parts of the world. However, their incidence and severity are low. Some species of rust appear to occur only where temperatures are moderate and rainfall is high. Other species predominate during hot dry seasons.

STEM ROTS

In many cassava-growing areas, continuous cassava planting is not possible and stakes must be stored for later propagation. Stored stems are attacked by three diseases that induce necrosis (CIAT, 1972). These diseases considerably reduce stake viability, directly and indirectly, by increasing dehydration and causing necrosis.

Although the three different causal agents have been recognized, the diseases these induce are not clearly differentiated in most cases. Macroscopically, the diseases look similar, particularly during their first developmental stages. Furthermore, more than one causal agent may be present, creating a syndrome.

The three diseases causing stem rots are stem necrosis caused by *Glomerella cingulata*, dry stem and root rot caused by *Diplodia* sp., and necrosis caused by an unidentified Basidiomycete (Lozano and Booth, 1979).

9. Stem Necrosis (*Glomerella cingulata*)

Importance

This disease is the most common of the three that induce rots or necrosis in stored cassava stems. It also attacks residues of old stems left in cassava fields.

Symptoms

Necrosis of stored stakes appears first at the ends and then progresses slowly towards the middle, before disseminating to all stems (**Figure 9**). The disease occurs as a black discoloration of vascular bundles. It then develops surface blisters that later break, exposing groups of black perithecia in well-developed stromata.

Etiology

The causal organism appears to be *Glomerella cingulata* (Commonwealth Mycological Institute, 1979, personal communication). Ascospores are hyaline, unicellular, and slightly curved. Infection probably occurs through wounds and is favored by high environmental relative humidity.

The relationship between this fungus and *Colletotrichum* sp., which causes anthracnose in cassava, has still not been determined. For example, the appearance of two types of symptoms may be due to two different states of the same agent rather than of two agents.

10. Dry Rot of Stem and Roots (*Diplodia* sp.)

Importance

This disease attacks stored cassava planting materials and residue stems left in the field. Its occurrence is not as common as necrosis caused by *Glomerella* spp.

Symptoms and epidemiology

The disease has two phases. The first is when root rot starts when soils are infested or when stakes from diseased plants are used. Symptoms, similar to those induced by root pathogens consists in sudden plant death caused by root deterioration.

The second phase includes stem rot caused by systemic invasion of the fungus from the roots or by penetration through wounds. The disease is characterized by black discoloration and necrosis of the vascular bundles, which extend from the infection sites,

i.e. wounds in the stem. In the epidermis, they appear as blisters under which the stem's internal tissues are discolored black or dark brown. The blisters break, showing confluent masses of black pycnidia (**Figure 10**). Gum may be excreted, and partial or total wilting occurs. Dieback may also occur.

The pathogen disseminates across great distances through stakes from infected plantings. Within the same crop, dissemination is by wind and rain during fungal fructifications, use of infested tools and irrigation water, and land preparation for later plantings.

Etiology

The causal agent of dry rot of stem and root is *Diplodia manihotis*. In both the host and laboratory cultures, this organism produces pycnidia that erupt through the stem or root surface, becoming confluent, stromal, and ostiolate. The conidiophores are short and simple, producing dark two-cell conidia that are slightly elongated on reaching maturity. Infection is believed to occur through wounds, and is favored by high environmental relative humidity.

Management and control

To control the disease, the cassava crop should be rotated with non-susceptible crops such as maize or sorghum, particularly when incidence is more than 3%. Planting stakes from healthy crops should be used and tools disinfected. Planting materials should be selected and handled carefully both before and after storage. Only viable cuttings or buds should be planted. One recommendation is to immerse cuttings in a solution of captan (3 g/l) and benomyl (3 g/l) for 5 min. Captan may be replaced by copper oxychloride.

ROOT ROTS

Root rots in cassava are important where soils are poorly drained or where excessively rainy seasons occur. In early growth, many microorganisms are capable of inducing not only root rots in young cassava plants, but also in the storage roots of mature plants. Although several root diseases have been reported, little information exists about them. Not even the symptoms are well described.

Usually, infection kills young plants at germination or shortly afterwards. Infection in plants older than 4 months may result in partial or total wilt, depending on whether the root rot is soft or dry. Once invaded by one or more primary pathogens, infected roots may be invaded by a wide spectrum of other micro-organisms. These are usually the otherwise weak saprophytic parasites, which become capable of degrading root tissues and masking the identity of the primary causal agent. The resulting root rots therefore appear to have the same syndrome of symptoms.

Pathogens causing root rots include *Phytophthora* spp., *Fusarium* sp., *Scytalidium lignicola, Rosellinia* spp., *Sclerotium* sp., and *Fomes lignosus* (Ferdinando *et al*., 1968; Jennings, 1970; Pereira, 1998; Viégas, 1955).

Some of these diseases often develop when cassava is planted immediately after woody crops such as coffee. Soils of such crops are infested with pathogens that attack

ligneous plants such as cassava. These pathogens may be fungi or bacteria that cause root deterioration, either as the crop grows or after harvest when roots are stored.

Control measures for these diseases are similar, the best comprising cultural practices such as good drainage, selection of loose-textured soils, crop rotation, early harvest, and avoiding soils prone to flooding. Treatments with fungicides may help establish the crop, preventing root rots from attacking during the crop's first months. Ridomil® (2.5 kg/ha), applied to the soil, and foliar applications of Alliette® (0.4 kg/ha) have shown good results. Fungicides based on plant extracts, oils, and cytokinins help control soil fungi, while offering a non-polluting organic alternative. Resistant varieties have also been reported (Castaño, 1953; CIAT, 1998; Drummond and Gonçalves, 1957; Fassi, 1957; Müller and De Carneiro, 1970; Sánchez, 1998).

11. Root Rot or "Black Rot" (*Rosellinia* spp.)

Importance

This disease has been reported in many cassava-growing regions with heavy, poorly drained soils that have a high content of organic matter. It is also found in cassava crops planted after forest crops or ligneous perennial species (Castaño, 1953; Viégas, 1955). The disease has also been called "black rot" because of the characteristic black color of infected tissues and root cankers.

In Colombia, dry rots are found in the coffee belt and in crops planted where coffee, cacao, or *guamo* (a shade tree used in coffee plantations) had previously been grown.

Symptoms and epidemiology

Initially, the root epidermis is covered with white rhizomorphs that later become black (**Figure 11**). Internally, infected tissues of bulked roots are slightly discolored and exude liquid on pressure. The black mycelial bundles penetrate the tissues, where they grow, forming small cavities that contain mycelium of an off-white color. The infected roots have a characteristic odor of decaying wood.

Etiology

Rosellinia necatrix, the perithecial state of *Dematophora necatrix*, is the causal agent of this disease (Castaño, 1953; Viégas, 1955). This fungus induces root rot in other ligneous and herbaceous plants (Castaño, 1953; Viégas, 1955). However, very little information is available on the epidemiology of the fungus in cassava. Its sexual state is generally believed to occur only very rarely (Castaño, 1953). Other *Rosellinia* species also attack cassava.

Management and control

Although the disease has not been reported in young plants, the recommendation is still to avoid selecting planting materials from infected crops.

Rotate with grasses whenever the incidence of plant death or root rot reaches 3%
Eliminate infected cassava residues and/or litter from perennial trees (e.g., trunks and decaying branches)
Plant in light-textured soils
Improve soil drainage

Treat by solarization, exposing the soil to the sun for three months
Chemical control with Topsin (thiophanate-methyl) at 2 g/l of commercial product and applied to the soil before planting
Applications of Sincocin (plant extract) to the soil at 1 l/ha are recommended. Stakes may also be immersed in a solution of the product at 1%.

12. Root Rot (*Sclerotium rolfsii*)

This disease commonly occurs in young stakes and mature roots, covering affected parts with a cottony mat. It has been reported only in Latin America (CIAT, 1972; Ferdinando *et al*., 1968). The white mycelium, which is found in infected roots or towards the base of stems, is also disseminated through the soil. This mycelium can, sometimes, penetrate roots through wounds, causing subsequent rot. Although it is rarely lethal to young plants, this fungus may cause a high incidence of root necrosis in a plant.

The disease is caused by *Sclerotium rolfsii*, a common soil organism but a weak pathogen. It has a white mycelium of cottony appearance. It also produces numerous round sclerotia, which characteristically form in the host or laboratory cultures.

13. Cottony Cassava Rot (*Fomes lignosus*)

Although this disease is known in Latin America, it is currently of minor importance. The disease is identified by the presence of a mass of white mycelium under the cortex of bulked roots and by the presence of white mycelial threads that look like cotton fibers covering part or all the epidermis of infected roots to the base of stems. Internally, the infected tissues look dehydrated and have a characteristic odor of decaying wood. Young plants may become infected and sometimes suffer sudden wilting, defoliation, and root necrosis.

The organism causing the disease is *Fomes lignosus* (IITA, 1972; Jennings, 1970).

DISEASES CAUSED BY PSEUDO-FUNGI

14. Root Rots (*Phytophthora* spp.)

Importance

Root rots are a very common problem in cassava production, causing yield losses that may be as high as 80% of total production.

Distribution

Root rot caused by *Phytophthora* spp. affect cassava in different agroecological areas in Africa (Fassi, 1957), tropical America (Müller and De Carneiro, 1970), and India (Johnson and Palaniswami, 1999). In Nigeria, Cameroon, and Benin, the pathogens causing root diseases of economic importance include *Sclerotium rolfsii*, *Botryodiplodia theobromae*, *Fomes lignosus*, *Rosellinia necatrix*, *Rhizoctonia solani*, *Phytophthora* spp., and *Fusarium* spp. (Hillocks and Wydra, 2001).

Recent reports mention that cassava root rots may cause losses between 5% and sometimes 100% in Latin America, Asia, and Africa, specifically, in Colombia, Brazil (Wania Fukuda and Chigeru Fukuda, 1996; EMBRAPA, Brazil; Fernando Takatsu, 1996, University of Brasília, Brazil, personal communications), Cuba (Maryluz Folgueras, 2002, INIVIT, Cuba, personal communication), Mexico (Luis Fernando Cadavid, 2005, CLAYUCA, personal communication), India (James George, 2004, CTCRI, India, personal communication), Uganda (William Serubombwe, 2003, NARO, personal communication), Nigeria, Kenya, Indonesia, Ghana, Ecuador, and probably in many other countries.

In Asia, root rots have recently been reported in Nondindang disctrict of Buriram province, and in Khonburi district of Nakhon Ratchasima province in Thailand, both areas characterized by sandy loam soils. Varieties showing the symptoms are Rayong 5, Kasetsart 50 and Huay Bong 60 (Álvarez, 2009, personal communication). The disease was also observed at the Rayong Field Crops Research Center, affecting Huay Bong 80 **(Figures 12** and **13).** Cassava root rots have also been reported in Vietnam.

In India, *Phytophthora palmivora* is emerging as a serious threat to cassava in several industrial areas of Tamil Nadu, where it is endemic. Crop losses are as high as 50%. Differential reaction of cassava varieties to infection by *Phytophthora* has been observed (Edison, 2002).

Symptoms and epidemiology

Phytophthora drechsleri macerates the root parenchyma, causing a penetrating odor and changing root color to cream (**Figure 14A**). *P. tropicalis* has been isolated from crops in Colombia (**Figure 14B**). In the State of Sergipe (Brazil), in 1976-1979, *P. drechsleri* was found to cause rot in the neck and roots, irreversible wilting of aerial parts, and defoliation (Souza Filho and Tupinamba, 1979) (**Figure 14C**). In contrast, *P. nicotianae* var. *nicotianae* shows little pepstatin activity. The odor is mild, with brown discoloration (Soto *et al*., 1988). Root attack by *P. drechsleri* leads to leaves falling and branch tips drying up before the plant dies (Figuereido and Albuquerque, 1970). *Phytophthora nicotianae* also causes a similar leaf blight in cassava (Erwin and Ribeiro, 1996; Lima *et al*., 1993).

Etiology

Farmers widely believe that root rots are caused by excess water in the soil. However, a study conducted in different edapho-climatic areas of Colombia showed that different *Phytophthora* spp. form the major cause of cassava root rots (Sánchez, 1998). Other pathogens also causing root rots include:

Fomes lignosus
Sclerotium rolfsii
Armillariella mellea
Fusarium spp.
Rhizoctonia sp.
Rhizopus sp.
Rosellinia necatrix (Lozano and Booth, 1979)
Pythium chamaehyphon (GenBank accession AY745748; CIAT, 2004)

Eleven species of *Phytophthora* have been reported as causing root rot. These are:

P. arecae (Coleman) Pethybridge (Alvarez *et al*., 1997b)

P. capsici Leonian (Lima *et al*., 1993)
P. citricola (CIAT, 1999, 2000)
P. cryptogea Pethybr. & Lafferty
P. drechsleri Tucker (Figueiredo and Albuquerque, 1970; Muller and De Carneiro, 1970)
P. erythroseptica Pethybridge (Fassi, 1957)
P. meadii (Barragán and Alvarez, 1998)
P. melonis (GenBank accession AY 739021; CIAT, 2000; 2004)
P. nicotianae Breda de Haan var. *nicotinae* (Dastur) (Soto *et al*., 1988)
P. palmivora (Johnson and Palaniswami, 1999 ; Álvarez *et al*., 2002)
P. tropicalis (GenBank accession AY 739022; CIAT, 2000; 2004)

The genetic diversity of these pathogens is broad and was determined through studies in Colombia with 80 isolates obtained from roots, young stems, and soils from 19 municipalities. These studies included the pathogen's pathogenicity, virulence, morphology, and molecular analysis of the internal transcribed spacer (ITS) region of the pathogen's ribosomal DNA. Eleven genetic groups were identified through PCR-RFLP (Alvarez *et al*., 1997a; 1997b; 2000; Sánchez, 1998). *Phytophthora tropicalis* was identified through sequencing of the ITS region of ribosomal DNA and isoenzymes, showing it to be genetically similar to *P. capsici* (CIAT, 2000). An isolate was obtained from cassava roots in Barcelona, Quindío, Colombia. *P. palmivora* was isolated from cassava roots at CIAT, Valle del Cauca, Colombia.

Integrated Disease Management

The integrated management of root rots includes the use of varietal resistance and/or cultural practices.

Varietal resistance.

A principal tool for managing root rots caused by various *Phytophthora* species is the use of varietal resistance. Various examples exist of the successful adoption of cassava clones resistant to *Phytophthora* spp. In 1990, the Brazilian Agricultural Research Corporation (EMBRAPA) and the Agricultural Research Center for the Humid Tropics (CPATU) released two cassava clones resistant to root rots: cvs. Mae Joana (IM-175) and Zolhudinha (IM-158). Both clones came from the State of Amazonas and are planted in the várzea ecosystem (a type of floodplains) of northern Brazil. The adoption of these clones, together with the application of appropriate cultural practices, increased root yields by more than 80% in this region (Lozano, 1991a).

With the MD-33 and Pao clones, high yields and resistance to root rot caused by *P. drechsleri* were obtained (Mendonça *et al*., 2003). Pereira (1998) reported resistance to *P. drechsleri* in seven cultivars from a group of 31 evaluated. Barragán and Alvarez (1998) reported 15 resistant genotypes from a group of 60 elite genotypes evaluated. Llano (2003) reported six individuals from a family of 126 genotypes, with high resistance to *P. tropicalis*, *P. palmivora*, and *P. melonis*. Although harvesting roots 14 months after planting resulted in increased yield it also demonstrated a higher incidence of root rots, thus showing that root rot incidence varies according to clones and harvest time.

In a participatory research study, indigenous communities of the Colombian Amazon adopted cassava clones resistant to *Phytophthora* spp. (Llano and Alvarez *et al*.,

2008; Llano *et al*., 2001). These clones were selected in the laboratory (harvested roots) and greenhouse (stems) from 700 genotypes provided by EMBRAPA and CIAT.

To obtain reliable information on the genetics of such a complex disease, Takatsu and Fukuda (1990) concluded that standardized methods were needed for inoculating and evaluating resistance to each cassava root rot pathogen. CIAT and the National University of Colombia in Palmira identified cassava clones resistant to *P. nicotianae* var. *nicotianae* by first inoculating bulked roots of plants that were 10 to 12 months old. They then added a suspension of the fungus to a nutritive solution in which 45 day-old seedlings were growing. Seedling roots were colonized by the pathogen. The inoculated roots were evaluated in terms of the percentage of the pathogen's colonization of cortical and parenchymatous tissues.

Inoculated bulked roots demonstrated variation in the severity of symptoms, depending on whether they came from resistant or susceptible clones. The inoculation method was easier to carry out, less expensive, and with faster results than the seedling method. No correlation was found between the two inoculation methods (López and Lozano, 1992).

Cassava seedlings planted in soil were also evaluated. The soil had previously been inoculated with a suspension of each of zoospores, oospores, or chlamydospores applied separately (Lima *et al*., 1993). Each inoculum type caused wilt and seedling death.

In 1995, Lima and Takatsu (1995) published the reactions of 13 cassava clones that had been stem-inoculated with three isolates of *P. drechsleri* in the greenhouse. The isolate with the most virulence was inoculated into roots in the field. To inoculate roots without harvesting them, inoculum was deposited in a small wound. The correlation between inoculated plants in the screenhouse and roots inoculated in the field was +0.24.

In other studies (Loke, 2004), several biochemical and morphological markers, and leaf resistance were identified for preselecting clones for resistance to *P. tropicalis* in cassava populations, based on (1) reduced area of the parenchyma with the presence of scopoletin in roots after harvest; (2) a high relationship between iron and manganese; and (3) resistance in leaves 72 hours after inoculation. Scopoletin is a coumarin that is found in very low concentrations in fresh roots, but which increases considerably after harvest. This substance is easy to quantify in roots, using ultraviolet light, and is related to the cassava root's susceptibility to post-harvest physiological deterioration.

Loke (2004) also demonstrated the benefits of using an index of resistance to *P. tropicalis* that includes molecular markers. The objective of this index is to select genotypes with durable resistance, based on a large diversity of resistance or defense mechanisms.

Several studies to identify the genetic base of resistance to *Phytophthora* have been conducted. A correlation of +0.31 between resistance during (in the peel, both epidermis and subepidermis) and after penetration (in the parenchyma) was observed for 25 cassava clones. This findings indicate that these forms of resistance are moderately associated (Corredor, 2005; Loke, 2004). Alvarez *et al*. (2003c), Llano *et al*. (2004), and Loke (2004) evaluated the cassava K family (150 F_1 individuals from the cross TMS 30572 × CM 2177-

2), inoculating root fragments. Nineteen QTLs were identified as associated with resistance to different species of *Phytophthora* and *Pythium*, three of which explained between 8.3% and 11% of phenotypic variance.

Those QTLs that were expressed were also found to vary from one cropping cycle to another, depending on prevailing environmental conditions. Minor genes were demonstrated as controlling resistance to *P. tropicalis*, *P. melonis*, and *P. palmivora*, with a high genotype-by-environment interaction existing. Although the population showed differences within its genetic base for resistance to *Phytophthora*, levels of resistance were not sufficiently high for use in improvement programs. Hence, identifying contrasting parents for the disease would be useful, as well as developing new populations for determining QTLs (Llano *et al*., 2004; Loke *et al*., 2004).

To identify genomic sequences in cassava that are homologous with genes of resistance to diseases of different plant species, two cassava families were evaluated for their resistance to *P. tropicalis* (GenBank accession AY 739022), *P. melonis* (GenBank accession AY 739021), and *P. palmivora*, all causal agents of root rot. Two strategies were used to search for genes for resistance: (1) hybridization with probes for maize and rice, using RFLP; and (2) amplifying conserved regions of DNA, using the degenerate primers NBS and Pto kinase. Three cassava clones resistant to *P. tropicalis* and *P. palmivora* were used, obtaining clones that were sequenced and homologized with known genes of resistance.

With hybridization, cassava demonstrated very low homology with the monocotyledon genes tested. Twenty-eight NBS and 2 Pto kinase clones were obtained, of which 14 showed homologous sequence with resistance gene analogs (RGAs) and NBS-LRR (GenBank accessions: AY730038, AY730040, AY730041, AY737490, AY745762, AY745763, AY745764, AY745765, AY745766, AY745767, AY745768, AY745769, AY745770, and AY745771). Four of these showed an open reading framework (ORF) with conserved motifs in the nucleotide-binding site (NBS) region, which means they were considered to be RGAs. Altogether, three classes of RGAs were identified, none of which showed association with resistance to *Phytophthora* (Llano *et al*., 2004).

Cultural practices

The best cultural practices for the integrated management of root rots are summarized below:

- Selecting an appropriate, well-drained, and moderately deep soil. If the land is flat and soils are clayey, planting should be done on ridges.
- To catalyze resistance, fertilizers should be applied in drench form, using potassium sources, and/or as foliar sprays, using potassium phosphites.
- If rot incidence reaches 3%, the cassava crop should be rotated with cereals or grasses, at least once a year.
- Eradicating diseased plants by removing infected roots from the field and burning them.
- Selecting healthy plants to obtain clean planting material. Where the farming area is infested, then stakes should be treated with metalaxyl at 0.3 g a.i./l.
- Treating stakes in hot water at 49ºC for 49 min is an alternative to chemical treatment (Alvarez *et al*., 2003d).

- Immersing stakes in a suspension of *Trichoderma harzianum* and *T. viride* at 2.5×10^8 spores/l, and later applying the same suspension in drench form (CIAT, 2006; 2007).

The biological control of root rots with isolates of *T. harzianum* and *T. viride* is promising (Bedoya *et al*., 2000; CIAT, 2006; 2007; Edison, 2002). Field trials in different agroecological zones of Colombia have shown that soil inoculated with strains of these types of *Trichoderma* will increase cassava yields (CIAT, 2001; 2006; 2007). Isolates of *Trichoderma* spp. were selected on the basis of *in vitro* antagonism, production of secondary metabolites that inhibit *Phytophthora* spp., and bioassays in screenhouses.

To identify practices of disease management that are feasible for indigenous communities in the northwestern region of the Amazon (Colombia), participatory research trials were established, with the women farmers making the evaluations. Soil amendments were incorporated. These were ash, organic matter (dry leaves), and a 1:1 mixture of both materials. Dosage was 200 g/plant. Cassava was also intercropped with cowpea (*Vigna unguiculata*), and planting stakes were selected from the middle part of healthy plants.

In these trials, cassava yields increased by 446% with applications of the ash and organic matter mixture. Where only ash was used, yields increased by 272%. Stake selection increased yield by 366%. Compared with traditional management, these practices reduced root rots by 100% (incorporation of the ash and organic matter mixture), 99% (association with cowpea), 94.2% (ash only), and 89.7% (stake selection) (Llano and Alvarez, 2008).

OTHER CAUSAL AGENTS OF CASSAVA ROOT ROTS

Other Fungal Root Rots

Other fungal species can induce root rots in cassava plants at different growth stages, but little information is available on these diseases and their importance. These root rots are caused by:

Armillariella mellea, which attacks both the stem base and roots of mature plants (Arraudeau, 1967; CIAT, 1972)
Phaeolus manihotis (Heim, 1931)
Lasiodiplodia theobromae (Vanderweyen, 1962)
Pythium sp. (CIAT, 1972)
Fusarium sp. (CIAT, 1972)
Clitocybe tabescens (Arraudeau, 1967)
Sphaceloma manihoticola (Bitancourt and Jenkins, 1950)
Rhizopus spp. (Majunder *et al*., 1956)
Rhizoctonia sp. (Gonçalves and Franco, 1941)
Aspergillus spp. (Clerck and Caurie, 1968)
Nattrassia mangiferae (Scytalidium sp.); Verticillium sp.; and Rigidoporus sp

Bacterial Root Rots

Some bacterial species belonging to the *Bacillus*, *Erwinia*, and *Corynebacterium* genera are also believed to cause soft rots and/or fermentation in bulked cassava roots

(Akinrele, 1964; Averre, 1967). Symptoms of these soft rots are similar and are frequently accompanied by fermentation. These agents probably penetrate roots through wounds produced by farmers during cultivation or by animals, insects, or fungi. They are frequently accompanied by other saprophytic microorganisms that help advance deterioration.

The causal agent of cassava bacterial blight (see below) can also induce necrosis, discoloration, and dry rot in the vascular tissues of infected roots (Lozano, 1973; Lozano and Sequeira, 1974).

Cassava Heart Rot

This physiological disorder damages bulked roots (Averrre, 1967). It occurs in moist and poorly drained soils in which roots present a dry internal necrosis that extends irregularly from the center to cortical tissues. This disorder is observed in only 10-20% of the roots of an infected plant. The larger and thicker roots are believed to be the most susceptible.

Postharvest Physiological Deterioration (PPD)

The cause of cassava roots' rapid deterioration after harvest is unknown, whether it results from physiological or pathological effects, or a combination of the two. Numerous microorganisms have nevertheless been isolated from deteriorated roots, with several being known to cause discoloration and rot.

DISEASES CAUSED BY BACTERIA

15. Cassava Bacterial Blight (CBB) (*Xanthomonas axonopodis* pv. *manihotis*)

Importance

Cassava bacterial blight (CBB) is regarded as one of the most limiting diseases of cassava production, as it can cause total crop loss in affected areas.

During the 1960s and 1970s, this disease caused major damage to the cassava crop. However, the application of integrated management programs, introduction of quarantine measures in some countries, and identification and planting of resistant varieties have led to its satisfactory control (Hillocks and Wydra, 2001; Lozano, 1986).

Distribution

Cassava bacterial blight has been known in Latin America since 1912, when it was reported in Brazil (Kemp, 2000). It spread to the cassava-growing regions of Africa and Asia in the 1970s (Boher and Verdier, 1994; Bradbury, 1986). In Latin America, the disease has been reported from most of the cassava-growing regions of Bolivia, Brazil, Colombia, Cuba, the Dominican Republic, Mexico, Panama, Trinidad and Tobago, and Venezuela (Cajar, 1981; Fukuda, 1992; Languidey, 1981; Lozano and Sequeira, 1974; Rajnauth and Pegus, 1988; A. Rodríguez, 1979; S. Rodríguez, 1992; Sosa, 1992; Trujillo *et al*., 1982).

In Asia, CBB has been observed during the rainy season in Thailand (**Figure 15**) as well as in many other countries but it is seldom very severe (Álvarez, 2009, personal communication). The disease was first observed in Taiwan before 1945 (Leu, 1976), and

has since been reported from Malaysia, Indonesia, Thailand (Booth and Lozano, 1978, Alvarez and Bellotti, 2009), Vietnam (Alvarez and Bellotti, 2009) and India (Cherian and Mathew, 1981).

Symptoms and epidemiology

Symptoms characteristic of CBB are small, angular, aqueous-looking leaf spots found on the lower surface of the leaf blade. Or symptoms may be leaf blight or brown leaf burn, wilt, dieback, and a gummy exudation in infected young stems, petioles, and leaf spots (**Figure 16**). The vascular bundles of infected petioles and stems are also necrotic, appearing as bands of brown or black color. Symptoms occur 11 to 13 days after infection (Lozano and Booth, 1979). Some susceptible varieties present dry and putrid spots around necrotic vascular bundles (Verdier, 2002).

The bacterium disseminates widely through stakes from infected plants, from one cropping cycle to another, and from one area to another. Within the crop, the principal means of dispersal are water splash from rain and contaminated tools. The movement of people and animals within the crop, especially during or after rain, may also help disperse the pathogen (Lozano, 1973).

Although the pathogen survives poorly in soil, this can be a source of inoculum if it is contaminated, as well as irrigation water, although in reduced proportions. The bacterium can survive epiphytically on many weeds, which serve as sources of inoculum if control is inadequate. Insects spread the disease over short distances.

The severity of CBB becomes greater when temperatures fluctuate widely between day and night. Hence, the disease is not important in areas of stable temperatures such as the Amazon Region, where the cloud cover does not permit marked fluctuations in temperatures.

Etiology

The causal organism, *Xanthomonas axonopodis* pv. *manihotis* (*Xam*), is a Gram-negative bacterium that is shaped like a slim cane. It is mobile by means of a polar flagellum. Its cells are not encapsulated, and the bacterium does not form spores.

The organism penetrates the host through stomas and wounds in the plant's epidermis. Infection is systemic, moving through the stems and petioles in xylem vessels and possibly also the phloem.

Xam can be detected, using the polymerase chain reaction (PCR), which amplifies a DNA fragment of 898 bp. This methodology permits detection to as low as 300 cfu/ml in leaves and stems infected by CBB (Verdier *et al*., 1998). When Verdier and Mosquera (1999) used the specific probe P898, they detected the bacterium in raw extracts of infected leaves and stems, and in cassava fruits and sexual seed. According to Verdier *et al*. (1993), pathogen diversity is narrow in Africa but broad in South America, cassava's center of origin.

Restrepo *et al*. (1996) reported that the diversity of the Colombian strains is very broad, at both pathogenic and genetic levels. Diversity is also high in Brazil (Restrepo *et al*., 1999) and Venezuela (Verdier *et al*., 1998).

Previous studies also revealed geographical differentiation among pathogen populations, according to ecozone. Evidence also exists of pathotypes moving within and between regions, probably because of movements of infected planting materials. In Colombia, analysis of pathogenic characteristics of *Xam* strains collected in three ecozones led to the definition of different pathotypes specific to each ecozone (Restrepo, 1999).

An analysis, using the AFLP technique, of the genetic variability of 85 *Xam* isolates from Brazil, Colombia, Cuba, and Venezuela distinguished three groups: (1) a cluster at a similarity level of 0.6 and formed of isolates from different localities in Colombia; (2) a cluster at 0.7 and comprising 81% of the Venezuelan isolates included in this study, and 4 Brazilian isolates; and (3) a cluster at 0.4 and formed by most of the Brazilian isolates, 3 isolates from Venezuela, 1 from Cuba, and 3 from Colombia. In this last group, clustering below the 0.4 similarity level also occurred, indicating great genetic variability within the Brazilian sites, possibly related to the equally high level of genetic diversity observed for the host plant (Sánchez *et al*., 1999). When new pathogen strains are introduced into a given area, the genetic diversity already found within the pathogen population is increased, thereby favoring the development of new pathotypes (Restrepo, 1999).

Integrated disease management

To control the disease, integrated management should be carried out, involving varietal resistance, cultural practices, and biological control.

Varietal resistance

The genetic control of CBB is the most efficient and economic method for the farmer, but the cassava cropping cycle is long, with a very low production of planting materials. Hence, the time involved in producing resistant varieties is very long. At CIAT, resistant varieties are identified through evaluations in the Eastern Plains and the Atlantic Coast, where the disease is acute and endemic. They are also evaluated in the greenhouse, under controlled conditions, with temperatures at 30°C and relative humidity at 95%.

In several greenhouse studies, plants of different cassava varieties were inoculated with 39 isolates from different regions of Colombia, Venezuela, and Brazil. Fifteen genotypes were identified as having high to intermediate resistance to CBB, i.e. scoring between 1.0 and 2.5 on a scale of severity from 1.0 to 5.0. These varieties included MEsc Fla 039, MEsc Fla 021, MBra 383, MCol 2066, CM 3311-4, CM 7772-13, and SM 1779-8 (CIAT, 1999; 2000; 2001; 2002b; 2003b).

Between 1995 and 2007, about 6400 cassava genotypes were evaluated in Villavicencio in the Eastern Plains of Colombia for their field resistance to CBB. Of these, 117 were identified as having partial resistance (CIAT, 2001; 2002b; 2003b; 2006; 2007).

In a 10 × 10 diallelic study, carried out in Villavicencio, with 45 families and 30 plants per family, the cassava genotype CM 4574-7 was identified as having high general combination ability. Its progenies showed increased resistance to CBB and Super elongation disease (SED) (Calle *et al*., 2005).

Tolerant varieties also exist such as MBra 685, MBra 886, ICA Catumare, ICA Cebucán, ICA Negrita, Vergara (CM 6438-14), CM 4574-7, and Chiroza. However, the disease has increased in severity in ICA Catumare, for which adequate selection of clean seed was not performed (Alvarez and Llano, 2002). Several genotypes have also been identified as having resistance to several pathotypes of the bacterium (Alvarez *et al*., 1999).

Zinsou *et al*., (2004) recommended the cassava genotype TMS 30572 for farmers in Benin, because of its high yield and relatively stable resistance to CBB across different environments. Kpémoua (1995) showed that resistance to *Xam* is associated with the production of phenolic compounds and the reinforcement of cell walls in the vascular system during early infection.

To determine the genetic control of resistance, 150 F_1 individuals of the cross TMS 30572 × CM 2177-2 were inoculated with the pathogen and evaluated for resistance under controlled conditions in the greenhouse. Five different *Xam* strains from the world's major cassava-growing areas were used in the study. Genetic analysis identified six genomic regions that control resistance to all *Xam* strains. One region controlled >60% of resistance to each of the strains CIO-1 and CIO-136. Two regions accounted for >70% of resistance to strain CIO-84. Another 80% of resistance to strains CIO-136 and ORST X-27 could be explained by 3 loci for each strain (Jorge *et al.*, 2000).

In three instances, the same genomic regions controlled resistance to two strains. A marker was obtained by Southern hybridization of a PCR amplification product from cassava, using heterologous primers designed from conserved regions of the *Xanthomonas* resistance gene in rice (*Xa21*). The region it marked explained 60% of phenotypic variance for resistance to strain CIO-136. A backcross population, derived from crossing members of the mapping population, has been developed and will provide more recombinations for fine mapping towards cloning resistance genes, and for studying intra-locus and inter-loci genetic interactions (Jorge *et al*., 2000).

A molecular genetic map of cassava was recently constructed from an F_1 cross of non-inbred parents. RFLP, AFLP, EST, SSR markers were used to map resistance to CBB. The F_1 cross was evaluated with *Xam* strains under both field and greenhouse conditions. Nine quantitative trait loci (QTLs), located on linkage groups B, D, L, N, and X, explained the phenotypic variance of the crop's response to *Xam* in the greenhouse.

Jorge *et al*. (2001) reported eight QTLs associated with resistance to CBB, and found changes in the expression of QTLs from one cropping cycle to another in the field, which could be related to changes in the pathogen's population structure. A QTL, located in linkage group D, was conserved over two cropping cycles and in resistance evaluations in the greenhouse. In a previous study, Jorge *et al*. (2000) showed that 12 different QTLs control resistance to five *Xam* strains.

Hurtado *et al*. (2005) detected the molecular marker, microsatellite SSRY 65, that could select resistant genotypes in a cassava family corresponding to the cross CM 9208-13 × MNga 19. Furthermore, the authors identified two RGAs of the NBS class through amplification with PCR, using two primers designed by Llano (2003). These RGAs could identify plant individuals that were resistant to the bacterium.

One approach to assessing cassava genetic diversity involves the structural analysis of genotypes resistant to CBB. Multiple correspondence analysis of AFLP data, using two primer combinations for cassava genotypes resistant and susceptible to two strains of *Xam,* elucidated the genetic structure of cassava germplasm resistant to CBB (Sánchez *et al*., 1999). Results revealed a random distribution of resistance or susceptibility, suggesting that resistance to CBB has arisen independently many times in cassava germplasm.

Phenolic compounds have been implicated in the resistance of cassava (*Manihot esculenta*) to xanthomonads. Cassava cultivars MCol 22 and CM 523-7 were inoculated with *Xam* and *X. cassavae*. CM 523-7 was susceptible to both pathogens, whereas MCol 22 was susceptible to *Xam* and resistant to *X. cassavae*. In the resistant interaction, no disease symptoms were observed in leaves. Bacterial growth was greatly reduced, and cell wall-bound peroxidase activity increased twofold, probably related to lignin deposition (Pereira *et al*., 2000).

Preformed putative defenses include copious latex production, which contains protease, β-1,3 glucanase, and lysozyme activities. ESTs from a latex cDNA library revealed a constitutive expression of many defense-related genes including chitinase, glucanase, and PAL. A cDNA-AFLP analysis of cassava leaves suffering a hypersensitive response to *Pseudomonas syringae* pv. *tomato* revealed that 78 genes, new to cassava, had expressed differentially. Homologs of a metalloprotease, glucanase, peroxidase, and ACC oxidase were all found to be upregulated. Pathogenicity determinants of *Xam* are being studied in the disruption of the *gum* biosynthesis gene (its EPS is produced copiously in plants) and the *pel* gene (pectate lyase appears as a single isoform) (Kemp *et al*., 2001).

RGAs were amplified as a means of elucidating the putative genes involved in cassava's defense response. For the cDNA-AFLP technique, of about 3600 cDNA fragments screened, 353 fragments were specific to a resistant variety. Sequence analyses showed significant homology with resistance genes, NPK-1 related proteins, senescence-related proteins, and other known proteins involved in disease resistance reactions.

Using degenerate primers, 12 classes of RGAs were identified in cassava. Screening a cassava cDNA library (root and leaf) with class-specific RGA probes also led to the identification of 16 expressed gene clones. Sequence analysis of clone L16 confirmed the constitutive expression of a protein that shares characteristics with previously reported resistance genes (Restrepo *et al*., 2001).

López *et al*. (2004a) identified 6046 unigenes and characterized a group of genes putatively involved in cassava's defense response to *Xam* infection. López *et al*. (2004b) identified the *RXam1* gene, homolog of *Xa21* from rice, in a 3600-bp DNA fragment. The gene is induced in the resistant variety (MBra 685), 72 hours after infection by *Xam*.

Cultural practices

The following practices are recommended:

- Use of healthy planting materials obtained from disease-free crops, plants derived from meristem culture, and by rooting buds and/or shoots

- Treating stakes by immersing them for 10 min in a solution of cupric fungicides such as copper oxychloride or Orthocide® (captan) at 3 to 6 g/l

- Immersion in an extract of citrus fruit seeds (Lonlife®)

- Heat treatment of stakes (Alvarez *et al*., 2008; CIAT, 2007), using hot water at 49°C for 49 min.

The incidence of CBB in untreated stakes was 37%, but dropped to 7% when treated with hot water. It dropped further to 0% when stakes were pretreated at 49°C for 10 min, 24 hours before being treated with hot water for 5 hours. Treatment with hot water did not, in practical terms, affect stake germination, reducing it by only 18% in the most prolonged treatment (Ramírez *et al*., 2000). The induction of enzymes that activate under stress conditions is probably responsible for conserving high stake germination, even after prolonged treatment in hot water.

Lozano (1986) also mentions the following practices for managing the disease:

- Planting at the end of rainy periods
- Crop rotation with grasses
- Planting barriers of maize to prevent dissemination by wind
- Improving soil drainage
- Weed control
- Fertilizers application, mainly sources of potassium
- Eradicating diseased plants
- Preventing the movement of people, machines, and animals from infected lots to healthy lots
- Eliminating infected materials after harvest by burning branches and stems
- Incorporating harvest residues into the soil

In field studies conducted in Benin and Togo by Wydra *et al*. (2001), locally and regionally well-adapted control measures for CBB were identified such as:

- Using locally preferred resistant varieties
- Intercropping with locally used crops
- Amending soils with local materials
- Fertilizer applications and recommendations on phytosanitary measures carried out to reduce disease

Complementary studies elucidated some mechanisms of resistance at the biochemical and genetic levels and molecular host-pathogen interactions.

New methods for detecting *Xanthomonas campestris* pv. *manihotis* (*Xcm*), using immunological and genetic techniques, were developed. Research results were partly verified under African conditions such as testing the cassava genome mapping population for reaction towards African strains to identify genetic markers and/or resistance related genes.

Biological control

Spraying with suspensions of *Pseudomonas putida* reduced the severity of damage caused by CBB, while cassava yields increased significantly (CIAT, 1985). However, this practice has not been adapted for farming conditions.

16. Bacterial Stem Rot (*Erwinia carotovora* pv. *carotovora*)

Importance

This disease is important for the damage it does to the quality and germinating capacity of planting stakes.

Symptoms

The disease is characterized by an aqueous and smelly stem rot or by medullary necrosis of the plant's ligneous parts (**Figure 17**). Infected plants show bud wilt. The stem's surfaces typically show perforations made by insects of the genus *Anastrepha* Schiner, which act as vectors for the bacterium. These orifices are easy to distinguish by the presence of dry latex, discharged as the stem is perforated. Diseased stakes used for planting will not germinate or they produce weak spindly plants, with a limited number of bulked roots (CIAT, 1972).

Management and control

Using healthy planting material
Planting with varieties resistant to the insect vector
Burning infected stems

Another bacterial disease is caused by *Erwinia herbicola*

17. Bacterial Stem Gall (*Agrobacterium tumefaciens*)

Symptoms and epidemiology

This disease generally appears on the lower parts of stems in plants older than six months. Characteristic symptoms, found on stem nodes, are galls that often become very large, presenting a proliferation of buds on the epidermis (**Figure 18**). Infected plants may become weak and spindly, and in the early days of infection, suffer dieback to as far as major galls. A single plant could have several galls on a stem and even along lower branches (Lozano *et al*., 1981)

The disease is usually initiated by infested soil being rain-splashed onto wounds caused by natural defoliation in stems of the plant's lower parts.

Management and control

Control is achieved through rotation with another crop when more than 3% of the planting is infected; disinfecting machetes with 2% sodium hypochlorite; always using planting stakes from healthy crops; and burning diseased materials within the crop (Lozano *et al*., 1981).

DISEASES CAUSED BY PHYTOPLASMAS (previously known as mycoplasma-like organisms or MLOs)

18. Cassava Frogskin Disease (CFSD)

Importance

Cassava frogskin disease (CFSD) is an economically important disease affecting cassava roots. It was reported for the first time in 1971, in the Department of Cauca, southern Colombia. Its origin appears to be the Amazon Region of Brazil or Colombia (Pineda *et al*., 1983).

Frogskin disease directly affects root production, causing losses of 90% or more. Symptoms consist of small, longitudinal fissures distributed throughout the root. As roots increase in diameter, the fissures tend to heal, giving the injuries a lip form. The root cortex or epidermis appears cork-like and peels off easily. Depending on the severity of symptoms, the depth and number of lesions increase until the root becomes deformed (Alvarez *et al*., 2003a; Pineda *et al*., 1983).

Distribution

In the 1980s, the disease occurred in most cassava-growing regions of Colombia and has continually spread. It has now been reported in Brazil, Costa Rica, Panama, Peru, and Venezuela (Calvert and Cuervo, 2002), Nicaragua, and Honduras. In Venezuela, it was reported for the first time in the States of Barinas and Aragua, with incidences between 11.4% and 14.3%, in cassava stakes grafted with 'Secundina', a variety used to diagnose the disease (Chaparro and Trujillo, 2001).

Symptoms and epidemiology

Frogskin mostly attacks cassava roots, reducing their diameter, but some varieties may also show symptoms in leaves such as mosaic, chlorosis, curling, and/or curvature in leaf margins (**Figure 19A**). However, these symptoms are difficult to distinguish under field conditions, and may be confused with damage from mites, thrips, viruses, and micronutrient deficiencies, or they can be masked when temperatures are >30°C.

Characteristic CFSD symptoms in the roots include a woody aspect and the thick, cork-like peel, which is also fragile and opaque. The peel also presents lip-like slits that may join to create a net-like or honeycomb pattern (**Figure 19B and C**). When roots do not bulk adequately (**Figure 19D**), the stems tend to be thicker than normal. In contrast, the roots of healthy plants are well developed, with thin, brilliant, and flexible peel.

Molecular tests, carried out on plants of cassava and pink vinca (*Catharanthus roseus* (L.) G. Don) after transmission trials with dodder (*Cuscuta* sp. L.), detected the presence of phytoplasmas associated with the 16SrIII group. Graft transmission could transfer phytoplasmas from infected to healthy plants (CIAT, 2005).

Insects were collected to identify the vector or vectors of the phytoplasma causing the disease. A homology of 90% was found among sequenced fragments from tissue of the insect *Scaphytopius marginelineatus* Stål (Hemiptera: Auchenorrhyncha: Cicadellidae) and from tissues of two cassava varieties.

Etiology

A phytoplasma was successfully detected and identified in CFSD-infected cassava roots, leaves, midribs, petioles, and peduncles of susceptible varieties. A nested PCR assay was used with the specific primers R16mF2/R16mR1 and R16F2n/R16R2. To classify the phytoplasma, the universal primers P1/P7 and R16F2n/R2 were used to amplify the 16S ribosomal DNA gene. Fragments measuring 1.2 kbp were amplified from samples collected only from symptomatic plants. Sequence analysis of the cloned fragments showed that the phytoplasma was similar to *Cirsium* white leaf phytoplasma (CirWL; GenBank accession AF373106, 16SrIII [X-disease] group), with a 99% sequence homology.

The phytoplasma was not detected in healthy plants from the same varieties harvested in fields free of disease. These results point towards the possible role played by phytoplasmas in this disease (Alvarez *et al*., 2003a; CIAT, 2002a).

The technique of reverse transcriptase AFLP, used to associate markers with CFSD plants, revealed that one AFLP product consistently associated with infected plants. It was cloned and sequenced, with the finding that a virus from the Reoviridae family (reo-like viruses) was infecting the cassava. This virus is now associated with the disease (Calvert *et al*., 2008).

Cuttings from CFSD-infected plants in the greenhouse were taken, and rooted in deionized water with different doses of chlortetracycline. Inhibition of leaf symptoms caused by CFSD was successful in two experiments when 50 ppm of chlortetracycline were used, thus indicating that CFSD is not caused by a virus. Nested PCR also showed that phytoplasmas were present in leaves of infected plants when treated with 0 ppm of tetracycline (CIAT, 2003a).

Although the disease spreads mostly through infected stakes, the disease is believed to have insect vectors. Numerous homopteran species (e.g., planthoppers, tree hoppers, and froghoppers) were collected from cassava fields in nine departments and 17 sites in Colombia. Three genera—*Scaphytopius fuliginosus* Osborn, *Empoasca* sp. Walsh, and *Stirellus bicolor* Van Duzee (Hemiptera: Cicadellidae) – were the most frequently collected. These three species are known vectors of viruses and phytoplasmas for other crops. Based on the evidence of high homology (80%) between insect and phytoplasma detected in cassava, *Sc. fuliginosus* appears to be a potential candidate as the vector for CFSD (CIAT, 2003a). However, tests for transmission have not yet effectively confirmed this hypothesis.

Integrated disease management

To date, the disease is managed principally by using stakes from healthy plants. Heat treatment, followed by meristem culture, has been used to obtain plants free of CFSD. Grafting with the susceptible variety Secundina is useful for monitoring the effectiveness of the heat treatment (Flor *et al*., 2001). Treating stakes at temperatures of more than 55°C appears promising but needs adjusting to reduce losses by the consequent low germination of stakes.

Cassava fields with more than 10% of CFSD incidence (foliage, stakes, and roots) should be burned. Plant health surveillance and quarantine systems need to be strengthened to prevent the entry or mobilization of planting materials from areas with the disease.

Field and greenhouse studies carried out at CIAT have reported 30 genotypes with different levels of resistance. These findings were confirmed through the expression of leaf symptoms in grafts with variety Secundina (CIAT, 2003a; Cuervo, 2006). The use of tolerant varieties will be a useful tool in controlling this disease.

19. Witches' Broom

Importance

This disease, known as *superbrotamiento* in Spanish, has been reported in Brazil, Venezuela, Mexico, and Peru (**Figure 20**). Although its incidence is not significant, the percentage of witches' broom in affected plantings is much higher than that of other diseases caused by American phytoplasmas. Crop losses can reach 80% (Lozano *et al*., 1981). In Asia, a new cassava disease was observed in Quang Ngai province of Vietnam (**Figure 21**). Typical symptoms similar to witches' broom are widespread in southern Vietnam, in Plangyao, Chacheoengsao, Thailand and also in the Philippines (**Figure 22**). The disease may seriously affect yields and the availability of clean planting material

Symptoms

Several symptomatologies exist:

1. Plants exhibit dwarfism and an exaggerated proliferation of buds; shoot proliferation and/or unusually rachitic branches growing from a single stake. Sprouts have short internodes and small leaves, but do not show deformation or chlorosis.
2. Proliferation of weak spindly sprouts on the stakes.
3. Stakes produce only a few dwarf and weak spindly sprouts that never reach normal size.
4. When the affected cassava is uprooted, the roots are thinner and smaller, with rough-textured skins, and drastically reduced starch content.

Etiology

The disease is transmitted mechanically and by the use of stakes from diseased plants (Lozano *et al*., 1981).

The transmission of cassava phytoplasmas by *Cuscuta* sp. into pink vinca was 100% positive. Symptoms appeared three weeks after implanting the host parasite into pink vinca in growth chambers at 18-20°C. No transmission was achieved with the insect *Scaphytopius fuliginosus*, even three months after exposure to feeding, whether cassava to cassava, cassava to vinca, or vinca to vinca (Valencia *et al*., 1993).

In Vietnam, disease recognition was carried out in the country's central and southern regions (Quang Ngai and Dong Nai provinces). Samples for diagnosing phytoplasmas were collected in southern Vietnam at Hung Loc Agricultural Research Center and from a farmer's plot in Dong Nai province, both sites being about 60 km from Ho Chi Minh City. In the samples collected in Thailand and Vietnam, phytoplasmas were

detected. Results of diagnosis confirm the association of symptoms (high bud proliferation, shoots with short internodes, and small leaves) with phytoplasmas.

Phytoplasmas were detected in roots, small leaves, and leaf veins showing symptoms. In the samples from Thailand and Vietnam no phytoplasmas of the 16SrIII group (reported in America) were found. However, we have evaluated only samples from the eastern and southern regions of these two countries, respectively. These results need to be confirmed. Through molecular tests based on the 16Sr gene, we were able to conclude that differences exist among the phytoplasmas detected in eastern Thailand and in southern Vietnam (Alvarez and Mejia, 2009).

Management

For disease prevention, using healthy planting materials and eliminating diseased plants in the field are recommended (Lozano *et al*., 1981). The disease is reduced by selecting stakes from healthy plants. Restrict the movement of cassava planting stakes, especially from infected areas and restrict the movement of related species such as jatropha. Varietal resistance also exists.

20. Antholysis (Phytoplasma)

Importance

Antholysis in cassava was observed in crops in southwestern Colombia in 1981 by Jayasinghe *et al.* (1983); it was severe in some experimental clones. However, this disease does not have economic importance and is only sometimes observed.

Symptoms

The disease appears in the inflorescence, with a characteristic virescence in the petals, which, instead of being their normal pink, become green. Hypertrophy of the petals is later observed and they become structures similar to leaves (phyllody). The floral racemes lose their normal appearance and resemble sprouts, giving this syndrome its name "antholysis" (*antho* – flower; *lysis* – dissolve, loosen) (**Figure 23**).

Infected flowers commonly exhibit a very swollen gynophore and develop internodes in the floral receptacle, a phenomenon known as apostasis. Furthermore, elongation of the receptacle occurs above the insertion of the pistil, with development of sprouts. Flower fertility is lost, resulting in nonfunctional flowers that abort prematurely. Affected plants do not present symptoms in other organs and, moreover, germination did not differ between infected and healthy stakes (Jayasinghe *et al*., 1983).

Etiology

By using an electron microscope, Jayasinghe *et al*., (1983) observed oval or spherical pleomorphic structures only in phloem tissues. Transmission is 100% by stakes. Under greenhouse conditions, symptoms of antholysis appear within one month of planting, contrasting with healthy plants, which take five months to flower.

Treatment with penicillin (500 to 1000 ppm) did not reduce symptoms, whereas tetracycline reduced antholysis by 90%. This sensitivity and detection by Dienes' stain confirmed that the causal agent is a phytoplasma (Jayasinghe *et al*., 1983).

Management

The disease is reduced by selecting stakes from healthy plants. Varietal resistance also exists.

DISEASES CAUSED BY VIRUSES

21. Cassava Mosaic Disease (CMD)

Importance

Cassava mosaic disease (CMD) was first described in 1894 in what is now Tanzania. The disease was later reported in many other countries of East, West, and Central Africa. It is now known to occur in all cassava-growing countries of Africa and adjacent islands, forming the major constraint to cassava production on this continent. Losses may be as high as 88% in susceptible varieties and 50% in varieties tolerant in the field (Edison, 2002; Obonyo *et al*., 2007). Calvert and Thresh (2002) reported disease incidence as being between 21% and 84% in 13 African countries, with the highest value in Kenya.

The disease also appears in India and Sri Lanka (Hillocks and Thresh, 2000), where it is gradually reaching alarming proportions in the cassava-growing states, causing losses as high as in Africa (Edison, 2002). Overall incidence of CMD is highest in the two main cassava-growing states in India: Kerala (23%) and Tamil Nadu (30%). It also appears in Andhra Pradesh (<1%) and Karnataka (5%), which are outside the main cassava-growing areas (Calvert and Thresh, 2002).

Symptoms

Symptoms begin in leaves as chlorotic areas intermixing with normal green tissue and creating a mosaic pattern and leaf distortion, leading to defoliation and severe stunting. In severe cases, leaves are reduced in size, twisted, and deformed (**Figure 24**). Plant height, stem diameter, petiole length, and leaf size are significantly reduced (Edison, 2002). Plants are stunted and young leaves abscise (Hillocks and Thresh, 2000). Symptoms are masked during hot dry months, making the identification of diseased plants impossible (Edison, 2002).

The disease is spread through infected planting materials or a vector, the whitefly (*Bemisia tabaci* Gennadius). It can also be transmitted through grafting.

Etiology

Cassava mosaic disease is caused by a begomovirus. An unusually virulent recombinant strain − the East African cassava mosaic virus of Uganda (EACMV-Ug) was associated with the severe CMD pandemic in East and Central Africa in the 1990s (Edison, 2002; Obonyo *et al*., 2007). Studies have identified several distinct but similar viruses: the African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV), Indian cassava mosaic virus (ICMV), and South African cassava mosaic virus (SACMV).

Recently, EACMV-UG occurred most frequently in the northernmost part of Angola (Uige province) immediately to the south of the Bas Congo region of the Democratic Republic of Congo, already known to be affected by the pandemic. These

findings significantly broaden the known geographical extent of the CMD pandemic and draw attention to the urgent need for the large-scale deployment of resistant cassava varieties, which have been used to reduce losses in pandemic-affected regions of East Africa (Lava Kumar *et al*., 2009)

In India, CMD is caused by the ICMV. This virus has been purified and was found to belong to the geminivirus group. Particles measure 18-24 nm in diameter. The rate of the virus' spread and crop losses depend on the time of infection, varietal susceptibility, climatic factors, and vector populations (Edison, 2002).

Antiserums of adequate titer strength can successfully detect even latent infections (Edison, 2002). Primer pairs, specific for ACMV and EACMV-Ug, are used to amplify fragments of DNA A of cassava mosaic geminiviruses (CMGs) (Obonyo *et al*., 2007).

A multiplex PCR was also developed for the simultaneous detection of ACMV and the East African cassava mosaic Cameroon virus (EACMCV) in cassava with CMD (Alabi *et al*., 2008). In Burundi most of the samples of cassava leaves exhibiting severe symptoms of CMD were found to be co-infected by three different begomoviruses (ACMV + EACMV + EACMV-UG). Multiple infections with begomoviruses within a same plant can drive to important evolution of the virus species and strains (Busogoro *et al*., 2008). A study on the molecular variability and distribution of cassava mosaic begomoviruses in Nigeria showed that ACMV was the dominant virus, forming 80% of all samples. The EACMCV was detected in single (2%) and mixed infections (18%) with the African cassava mosaic virus DNA A (ACMVA) (Ariyo *et al*., 2005). ACMV showed little variation in its genomic sequence in Côte d'Ivoire, while EACMV is more genetically diverse because of frequent recombinations of its two components (i.e. EACMV and EACMV-Ug) (Pita *et al*., 2001).

Studies on variability first showed that the EACMV-Ug virus arose from a recombination event between ACMV and EACMV. Mixed virus infections were frequent, resulting in a synergistic interaction (Legg and Thresh, 2003); this aspect of the cassava-CMD pathogen system has to be taken into consideration for a successful implementation of plant genetic resistance to control CMD.

Integrated disease management

Management comprises:

- Use of field-tolerant cassava varieties like H-97, H-165, and Sree Visakham in India.
- Selection of disease-free meristem-derived planting materials, followed by clonal multiplication with periodic screening and rouging of newly infected plants.
- Disease-free planting materials should be selected before the beginning of the hot dry season. These materials can be multiplied on a large scale at higher altitudes where whitefly populations are small or non-existent.
- Raising plants in the nursery at closer spacing before transplantation into the main field to prevent the primary spread of the disease in the main field.

- Adherence to strict phytosanitary practices such as timely harvesting, prompt disposal of crop residues, and eradication of self-sown plants and weeds that may harbor both the disease and vectors (Edison, 2002).

Resistance to CMD has been successfully incorporated into high-yielding cultivars of acceptable quality. CMD-resistant materials have been evaluated, and many promising clones have been selected in various countries in tropical Africa and India (Hahn *et al*., 1980). Levels of resistance have been effective in those countries. Currently, the seven cassava varieties highly recommended for cultivation in areas affected by cassava brown streak disease (CBSD; see below) and CMD of Tanzania are 'Kiroba', 'Kigoma Mafia', 'Nachinyaya', 'Kalulu', 'Kitumbua', 'Namikonga', and 'Naliendele'. In 2007, Zanzibar released another four new cassava varieties that are tolerant of CBSD, resistant to CMD, and which meet consumer preferences in markets (Manyong and Abass, 2007).

Even so, while some farmers could obtain CMD-resistant varieties, adoption rates are very low. Strong multiplication and dissemination efforts are required to encourage the adoption of CMD-resistant varieties (Obonyo *et al*., 2007).

Little use is made of insecticides to control the whitefly vector, but such measures are inappropriate anyway for a widely grown subsistence crop. Only limited attention has been given to other possible control measures such as the use of intercropping, crop disposition, or manipulation of planting date to decrease risk of infection (Thresh and Otim-Nape, 1994, cited by Hillocks and Thresh, 2000). Such measures merit consideration in the current search for integrated means of control that would make the most effective use of phytosanitation and resistant varieties (Hillocks and Thresh, 2000).

22. Cassava Brown Streak Disease (CBSD)

Cassava brown streak disease (CBSD) is a poorly researched viral disease that is of emerging prevalence and importance in the Great Lakes Region, and acknowledged as representing a significant threat to production and food security of small holders. The disease is caused by two variant strains of virus (Cassava Brown Streak Virus (CBSV)) have recently been described by whole genome analysis that have confirmed earlier reports of a 'Coastal' or 'Tanzanian' (CBSV) strain and a 'Highland' or 'Uganda' (CBSUgV) strain (Smith and Tomlinson, 2010). The two strains belong to a genus *Ipomovirus*, family *Potyviridae*. The disease affects both the yield and quality of the tuberous roots of cassava.

The history and current knowledge on CBSD have been reviewed by Hillocks and Jennings (2003). The disease was first reported and distinguished from cassava mosaic disease (CMD) in Tanzania during the 1930s. Soon after, the whitefly, *Bemisia tabaci* (Gennadius), was suggested as a possible vector. Successful transmission of CBSV by *B. tabaci* between cassava plants was achieved only recently in 2004 (Maruthi *et al.*, 2005).

Importance

Surveys for CBSD found the disease to be endemic in all East African coastal cassava growing areas from Kenya to the Ruvuma River that marks the border between Tanzania and Mozambique. It was reported to be widespread in coastal Kenya and in Mozambique where it was prevalent. Recent surveys have confirmed that the disease

occurs throughout the coastal strip of Lake Malawi. These gaps in knowledge on CBSV highlight the concern associated with the movement of cassava material infected with CBSVs. It is known that the main mechanism of spread of these viruses is with cassava planting stakes (Smith and Tomlinson, 2010). Symptoms resembling those of CBSD have been reported from Bas-Congo and Kinshasa Provinces of the Democratic Republic of Congo, but the virus has not been confirmed by diagnostics. The reasons for the restricted occurrence of CBSD, despite the distribution of *B. tabaci* throughout Africa and the considerable movement of cassava planting materials, remain unknown. Early studies led to the observation that CBSD could be found inland from the East African coast up to an altitude of 1,000 m above sea level (masl). Surveys conducted during the 1990s appeared to support that view. Although cassava is widely grown at altitudes above 1,000 masl in Tanzania, Malawi, and Mozambique, CBSD has not been reported from these areas. A nationwide survey of Tanzania in 1993 and 1994 showed CMD and *B. tabaci* in all parts of the country, but CBSD was found only in the lowlands bordering the Indian Ocean and Lake Malawi. Wherever the disease has been reported to be endemic, occurrences were confined to altitudes below 1,000 masl, and incidence increased with decreasing altitude. However, it has been known for some time that CBSD symptoms can be expressed at altitudes greater than 1,000 masl when infected cuttings have been planted. This occurred in Uganda when infected material was taken from Tanzania in 1934, but the disease was eradicated by destroying all plants showing symptoms. From that time until 2004, CBSD has not been prevalent in Uganda, although CBSD-like symptoms were observed on a few cassava plants at one site in central Uganda in 1994 (Alicai *et al.*, 2007).

Alicai *et al.* (2007) report a new outbreak of CBSD in Uganda and confirmation of the presence of CBSV by reverse transcription-polymerase chain reaction (RT-PCR) and nucleotide sequence analysis. CBSD is a major disease of cassava in eastern Africa, and because of its direct effect on the quality of tuberous roots, it constitutes a significant threat to food production. Their results highlight the possibility that other countries in the region previously unaffected by CBSD may be at risk of spread and increased prevalence of the disease.

Symptoms

There are a number of different symptoms in the CBSD syndrome. On leaves, the disease appears as a feathery chlorosis on either side of the smaller veins. There are several variants of this symptom, depending on cultivar, crop age, and weather conditions. Characteristic CBSD foliar symptoms normally occur only on mature leaves, and the young expanding leaves are symptomless. The economically damaging symptom occurs on the tuberous roots as a yellow/brown, corky necrosis in the starch-bearing tissues, and radial root constriction occurs in very severe infections. The necrosis begins as discrete areas, but in fully susceptible cultivars, it may affect most of the root, rendering the roots unfit for human consumption.

Symptoms of the disease are first seen as discoloration of the cassava leaves along the veins (**Figure 25**). In severe cases, the whole leaf looks blotchy with yellow-light green spots. The lower leaves are usually most affected but leaves are not deformed. In roots, symptoms may be external or internal or, sometimes, both. External symptoms include constrictions in the roots and/or pits in the surface bark. Underneath the bark the cortex is

necrotic (**Figure 25**). Internal symptoms are yellow to brown corky patches in the root pulp under the root cortex. Sometimes, blue to black streaks appear. Often, roots look healthy on the outside until they are cut open, when the streaks become evident (Manyong and Abass, 2007). Disease symptoms are highly variable and may be influenced by virus load (titre), variety (levels of tolerance) and environmental (temperature, rainfall, altitude, soil fertility) factors (Obonyo *et al*., 2010; Smith and Tomlinson, 2010).

In the more susceptible varieties, purple to brown lesions on the exterior surface of young green stem tissues may be observed. On stripping off the outer bark, the lesions can be seen to penetrate the cortex. Necrotic lesions appear in leaf scars after leaves are shed through normal senescence.

Root symptoms usually develop after foliar symptoms. The period between infection and the onset of root necrosis seems to be cultivar dependent. In some cultivars, root necrosis does not develop in infected cuttings until more than 8 months after planting, despite the earlier presence of clear foliar symptoms. In the most susceptible cultivars, where planting materials have derived from infected stock, root necrosis can become apparent at five months after planting (Hillocks and Thresh, 2000).

Etiology

Leaf samples showing typical CBSD symptoms were sent to UK for electron microscopy whereby virus particles were detected. The particles were elongate, flexuous filaments 650-690 nm long that contained “pin-wheel” inclusions, typical of potyviruses. The exact etiology of the disease remained a matter of speculation until the coat protein gene of CBSV was recently cloned and sequenced at the University of Bristol, UK. The virus has since been shown to be an ipomovirus, a whitefly-transmitted potyvirus (G. Foster 1997, unpublished data; Hillocks and Thresh, 2000). New diagnostics to CBSV have been developed that target together or independently the two CBSVs (Smith and Tomlinson, 2010). There are advantages to real-time PCR over conventional PCR in terms of sensitivity and avoidance of false positives. Real-time PCR has become available to Kenya, Tanzania and Uganda. (Smith and Tomlinson, 2010).

Very little information is currently available on the variability of different CBSV isolates. Only a few isolates have been examined, and these cannot be considered to be representative for CBSV in East and Central Africa as a whole. However, the University of Bristol team examined CBSV isolates from three varieties obtained in Tanzania and Mozambique. The isolates each elicited different symptom types in herbaceous indicator plants, but comparisons of sequences revealed only 8% differences in nucleotides and 6% differences in deduced amino acids (Legg and Thresh, 2003).

Integrated disease management

The basic approach to controlling CBSD is to select planting materials from symptomless mother plants. The stock’s health needs to be maintained by continued selection and roguing of any infected individuals that appear at sprouting. The success of this approach depends on the amount of disease in surrounding cassava and the rate of dissemination. The mechanism for dissemination is unknown for CBSV and the practicality of virus-free planting materials cannot yet be predicted. However, this measure may be worthwhile for areas with low disease pressure.

For areas with high disease pressure such as the Tanzanian coast and much of Mozambique, the release of virus-free planting materials needs to be combined with deployment of cultivars with some form of resistance. Local cultivars such as 'Nachinyaya' in southern Tanzania that apparently tolerate infection and are slow to develop root necrosis could be used. Surveys conducted in Tanzania have indicated that other cultivars also show varying degrees of resistance to or tolerance of CBSV (Hillocks and Thresh, 2000).

23. Cassava Common Mosaic Disease (CsCMD)

Cassava common mosaic disease (CsCMD) was first reported in southern Brazil. The disease has since been reported from other South American countries, Africa, and Asia. The disease has no known vector, and probably spreads through mechanical transmission. CsCMD is generally of minor importance, although it is prevalent in some areas like southern Brazil and Paraguay.

Symptoms

Leaves of cassava plants infected by CsCMD develop mosaic and chlorotic symptoms (**Figure 26**). On some infected leaves, dark and light green patches are delimited by veins. Symptoms are most severe during relatively cool periods; cassava grown in the subtropics of South America is most affected by the disease. Under these conditions, losses may be as high as 60% (Costa and Kitajima, 1972, cited by Calvert and Thresh, 2002).

Etiology

CsCMD is caused by the cassava common mosaic potexvirus (CsCMV). The CsCMV genome is single-stranded RNA for which the complete sequence is known. Vectors of CsCMV are unknown and the primary source of inoculum is infected planting materials. The virus is systemic in cassava and almost all stem cuttings are infected when obtained from an infected plant.

Management

Some practices can eradicate the disease or reduce it to a level of minor economic significance. Eliminating plants that express CsCMV provides adequate control. Only healthy plants should be selected as sources of planting materials. Cutting tools should be disinfected.

24. Cassava Vein Mosaic Disease (CVMD)

Importance

CVMD was reported in 1940 by Costa (cited by Calvert and Thresh, 2002). This disease has received little attention because symptoms are sporadic and generally less apparent by the end of the cassava growing cycle. CVMD is very common in the semi-arid zone of northeastern Brazil, with reports of its occurring in other regions of the country.

Symptoms

Leaf symptoms of CVMD occur in flushes. After an infected stake sprouts, the first four to six leaves express vein chlorosis, which appears as a chevron pattern or coalesces to

form ring spots. Leaf deformation and epinasty are common severe symptoms. Plants then appear to grow out of the infection and produce several symptomless leaves. These are followed by another series of leaves with symptoms. Except for the period just after sprouting, CVMD does not seem to affect plant vigor. The affected leaves senesce and fall prematurely from the plants, reducing leaf area. As the infected cassava matures, it is often difficult to see any leaves with mosaic symptoms.

Etiology

CVMD is caused by the cassava vein mosaic virus (CVMV), whose genome consists of dsDNA. The virus, a pararetrovirus, will probably be classified as a unique genus for the plant. The only known host is cassava and the primary mode of dissemination is in infected planting materials. Spread occurs within fields, which suggests that the virus has no vector.

Management

Virus-free planting materials should be used. Roguing infected planting materials may be effective if removal is done soon after sprouting.

OTHER PATHOGENIC VIRUSES

Cassava Virus X **(CsVX),** ***Cassava Colombian Symptomless Virus*** **(CCSpV)**, and other potexviruses infect cassava (Lennon *et al*., 1986, cited by Calvert and Thresh, 2002) (**Figure 27**). They were detected first in Colombia, and little effort has been made to determine if they occur elsewhere. However, CsVX was detected in Venezuela by Chaparro-Martínez and Trujillo-Pinto (2003). Calvert and Tresh (2002) also mention the ***Cassava American Latent Virus*** **(CsALV)** (Comoviridae: Nepovirus). Little is known of its distribution. As the three viruses named do not cause symptoms, their distribution or importance has not been determined.

Calvert and Thresh (2002) cite other viral diseases. These include the ***Cassava Ivorian Bacilliform Virus*** (unassigned), ***Cassava Kumi Viruses A and B***, and ***Cassava "Q" Virus*** reported in Africa; and the ***Sri Lankan Cassava Mosaic Virus*** **(SLCMV)** (Geminiviridae: Begomovirus); and ***Cassava Green Mottle Virus*** **(CGMV)** (Comoviridae: Nepovirus) reported in Asia.

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CHAPTER 12

DIAGNOSIS OF NUTRITIONAL PROBLEMS OF CASSAVA [1]

Reinhardt Howeler[2]

INTRODUCTION

If plant growth is not optimal and/or yields are low, and if other causes such as insects and diseases, drought, shade or cold have been ruled out, plants may be suffering from nutritional deficiencies and/or toxicities. Before effective remedial measures can be taken, it is essential to diagnose the problem correctly. This can be done in several ways, but the best diagnosis is usually obtained from a combination of different methods:

1. Observation of Deficiency and Toxicity Symptoms

Cassava has relatively low phloem mobility. As such, plants do not readily translocate nutrients from the lower to the upper leaves. Instead, when certain nutrients are in deficient supply, plants respond by slowing the growth rate, producing fewer and smaller leaves and sometimes shorter internodes. Leaf life is also reduced. As nutrients are not readily mobilised to the growing point, symptoms for N, P or K deficiencies, normally found in the lower leaves, tend to be less pronounced in cassava than in other crops. For that reason farmers may not be aware that plant growth is reduced because of nutritional deficiencies. Oftentimes, the initial diagnosis based on deficiency or toxicity symptoms needs to be confirmed by soil or plant tissue analyses or from experiments. Nevertheless, visual identification is a quick and easy method to diagnose many nutritional problems.

The various nutrients the plant needs also vary in their mobility in the phloem. Thus, N, P, K, Mg, Na and Cl are considered relatively mobile, so in case of insufficient supply of these nutrients, the plant will translocate these nutrients from the lower part of the plant to the growing point, resulting in deficiency symptoms appearing mainly in the lower leaves. In contrast, Ca and B are very immobile and will not readily translocate to the upper part of the plant, resulting in deficiency symptoms of these two nutrients being confined mainly to the growing points of both shoots and roots. Finally, S, Cu, Fe, Mn and Zn have intermediate mobility, so their deficiency symptoms can appear in various parts of the plant or throughout the plant.

Symptoms have been described and color photos have been included in several publications (Lozano *et al*., 1981; Asher *et al*., 1980; Howeler, 1981; 1989; 1996a; 1996b; Howeler and Fernandez, 1985). The symptoms of nutrient deficiencies and toxicities are briefly described in **Table 1,** while some symptoms are shown in the photos at the end of this chapter..

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[1] For color photos see pages 761-766.

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Table 1. Symptoms of nutrient deficiencies and toxicities in cassava.

Deficiencies	Symptoms
Nitrogen (N)	▪ Reduced plant growth ▪ In some cvs., uniform chlorosis of leaves, starting with lower leaves, but soon spreading throughout the plant
Phosphorus (P)	▪ Reduced plant growth, thin stems, short petioles; sometimes pendant leaves ▪ Under severe conditions 1-2 lower leaves turn yellow to orange, become flaccid and necrotic; may fall off ▪ In some cvs. lower leaves turn purplish/brown
Potassium (K)	▪ Reduced plant growth with excessive branching, resulting in prostrate plant type ▪ Small, sometimes chlorotic upper leaves; thick stems with short internodes ▪ Under severe conditions premature lignification of upper stems with very short internodes, resulting in zigzag growth of upper stems ▪ In some cvs. purple spotting, yellowing and border necrosis of lower leaves ▪ In other cvs. upward curling of lower leaf borders, similar to drought stress symptoms
Calcium (Ca) (seldom seen in the field)	▪ Reduced root and shoot growth ▪ Chlorosis, deformation and border necrosis of youngest leaves with leaf tips or margins bending downwards
Magnesium (Mg) (often seen in field)	▪ Marked intervenal chlorosis or yellowing in lower leaves ▪ Slight reduction in plant height
Sulfur (S) (similar to N deficiency)	▪ Uniform chlorosis of upper leaves, which soon spreads throughout the plant
Boron (B) (seldom seen in field)	▪ Reduced plant height, short internodes, short petioles and small deformed upper leaves ▪ Purple-grey spotting of mature leaves in the middle part of the plant ▪ Under severe conditions gummy exudate on stem or petioles (almost never seen in field) ▪ Suppressed lateral development of fibrous roots
Copper (Cu) (mainly in peat soils)	▪ Deformation and uniform chlorosis of upper leaves, with leaf tips and margins bending up- or down-ward ▪ Petioles of fully expanded leaves long and bending down ▪ Reduced root growth

Iron (Fe) (mainly in calcareous soils)	▪ Uniform chlorosis of upper leaves and petioles; under severe conditions leaves turn white with border chlorosis of youngest leaves ▪ Reduced plant growth; young leaves small, but not deformed
Manganese (Mn) (mainly in sandy and high pH soils)	▪ Intervenal chlorosis or yellowing of upper or middle leaves; uniform chorosis under severe conditions ▪ Reduced plant growth; young leaves small, but not deformed.
Zinc (Zn) (often seen in high pH or calcareous soils; also in acid soils)	▪ Intervenal yellow or white spots on young leaves ▪ Leaves become small, narrow and chlorotic in growing point; necrotic spotting on lower leaves as well ▪ Leaf lobes turn outward away from stem ▪ Reduced plant growth; under severe conditions, death of young plants

Toxicities	**Symptoms**
Aluminium (Al) (only in very acid mineral soils)	▪ Reduced root and shoot growth ▪ Under very severe conditions yellowing of lower leaves
Boron (B) (only observed after excessive B application)	▪ Necrotic spotting of lower leaves, especially along leaf margins
Manganese (Mn) (mainly in acid soils and when plant growth stagnates)	▪ Yellowing or oranging of lower leaves with purple-brown spots along veins ▪ Leaves become flaccid and drop off
Salinity (observed only in saline/ alkaline soils)	▪ Uniform yellowing of leaves, starting at bottom of plant but soon spreading throughout ▪ Symptoms very similar to Fe deficiency ▪ Under severe conditions border necrosis of lower leaves, poor plant growth and death of young plants

2. Soil Analysis

This method is advantageous in that problems can be detected before planting and, if necessary, lime and/or nutrients can be applied before plant growth is affected by the problem. Soil analyses are particularly useful for detecting P, K, Ca, Mg and Zn deficiencies, while soil pH will indicate whether Al and/or Mn toxicity or micronutrient deficiencies are likely to occur. Analysis for OM content is not very reliable in predicting N responses as high-OM soils may still produce a significant N response if N mineralization is slow, especially in very acid soils.

Representative soil samples should be taken in areas that appear to be uniform in terms of plant growth and previous management. About 10-20 subsamples are taken in zigzag fashion across the whole area. These subsamples are thoroughly mixed together and then about 300-500 g is air dried or dried at about 65°C in a forced-air oven. This compound sample is then finely ground, screened and sent to the lab for analysis.

Soil analyses usually determine the amount of available or exchangeable nutrient as this part of the total soil nutrient is best correlated with plant uptake. These "available" fractions are usually determined by shaking the soil sample with certain extracting solutions and determining the amount of nutrient in the extract. Different laboratories may use different extracting agents as there is no one method that is optimal for all soil types; thus results from one lab may differ from those of another. In interpreting the results, therefore, it is important to consider the methodology used.

Results of the soil analysis can be compared with published data obtained from correlation studies, which indicate either the "critical level" of the nutrient, as determined with a specific extracting agent or the nutrient ranges according to the particular nutritional conditions of the crop. The ranges are defined according to the various nutritional states of the plant, as shown in **Figure 1. Table 2** shows the ranges for soil nutrients determined for cassava.

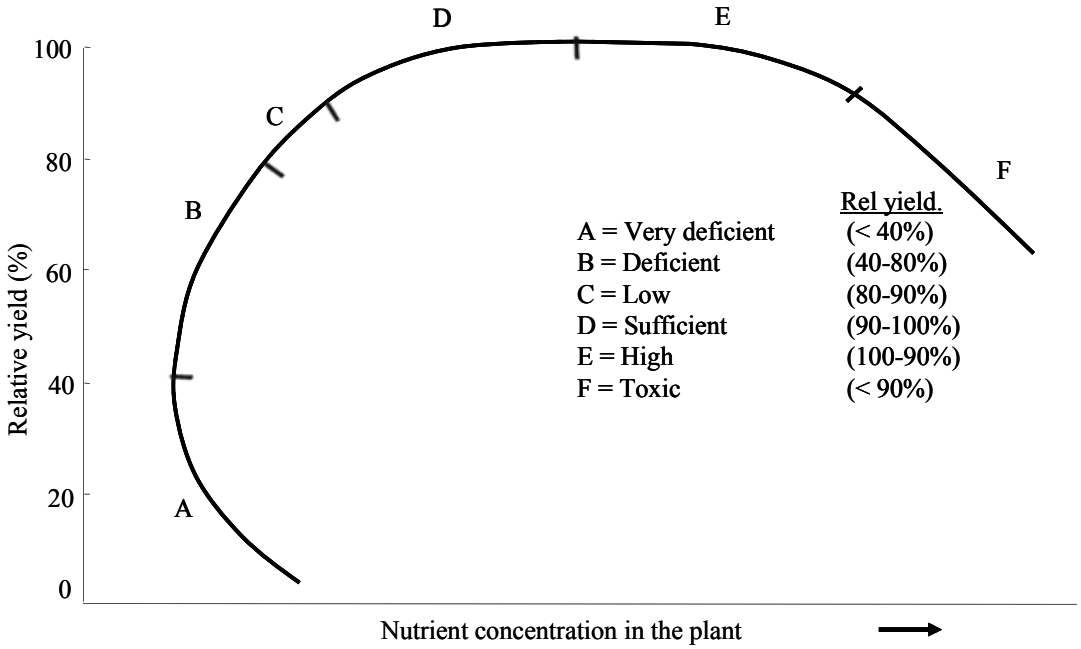

Figure 1. Relation between the relative yield or dry matter production of the plant and the concentration of the limiting nutrient in the soil or plant tissue. The curve is divided into six defined nutritional states, ranging from very deficient to toxic.

Table 2. Approximate classification of soil chemical characteristics according to the nutritional requirements of cassava.

Soil parameter	Very low	Low	Medium	High	Very high
pH[1)]	<3.5	3.5-4.5	4.5-7	7-8	>8
Organic matter[2)] (%)	<1.0	1.0-2.0	2.0-4.0	>4.0	
Al saturation[3)] (%)			<75	75-85	>85
Salinity (mS/cm)			<0.5	0.5-1.0	>1.0
Na saturation (%)			<2	2-10	>10
P[4)] (ppm)	<2	2-4	4-15	>15	
K[4)] (meq/100 g)	<0.10	0.10-0.15	0.15-0.25	>0.25	
Ca[4)] (meq/100 g)	<0.25	0.25-1.0	1.0-5.0	>5.0	
Mg[4)] (meq/100 g)	<0.2	0.2-0.4	0.4-1.0	>1.0	
S[4)] (ppm)	<20	20-40	40-70	>70	
B[5)] (ppm)	<0.2	0.2-0.5	0.5-1.0	1-2	>2
Cu[5)] (ppm)	<0.1	0.1-0.3	0.3-1.0	1-5	>5
Mn[5)] (ppm)	<5	5-10	10-100	100-250	>250
Fe[5)] (ppm)	<1	1-10	10-100	>100	
Zn[5)] (ppm)	<0.5	0.5-1.0	1.0-5.0	5-50	>50

[1)] pH in H_2O. 1:1
[2)] OM = Walkley and Black method.
[3)] Al saturation = 100 x Al(Al+Ca+Mg+K) in meq/100 g.
[4)] P in Bray II; K, Ca, Mg and Na in 1N NH_4-acetate; S in Ca phosphate.
[5)] B in hot water; and Cu, Mn, Fe and Zn in 0.05 N HCl+0.025 N H_2SO_4.
***Source**: Howeler, 1996a, b.*

The data in **Tables 2** and **3** were determined from many fertilizer experiments conducted in Colombia and in various Asian countries, as well as from reports in the literature. The data on ranges or critical levels were determined by relating the relative yield in the absence of a particular nutrient (yield without the nutrient over the highest yield obtained with the nutrient) with the corresponding available nutrient content in the soil.

Figure 2 shows an example of the determination of critical levels from NPK experiments conducted in nine locations in four Asian countries. A line was drawn visually through the points to show the relationship and to estimate the "critical level" of the nutrient or soil parameter. This critical level is normally considered as the concentration of the nutrient in the soil or plant tissue above which there is no further significant response to application of the nutrient (usually defined as corresponding to 90 or 95% of maximum yield). Critical levels for cassava were found to be about 3.2% for OM, 7 ppm for P (Bray II) and 0.14 meq/100 g for exchangeable K. The critical levels for P and K are close to those reported earlier in the literature **(Table 3)**. Those for available soil-P reported for cassava (4-10 ppm) are much lower than for most other crops (10-18 ppm), indicating that cassava will grow well in soils that are low in P and where other crops would suffer from P deficiency. This is due to the effective association between cassava roots and vesicular-arbuscular mycorrhizae (VAM) occurring naturally in the soil (Howeler, 1990) (see Chapter 19).

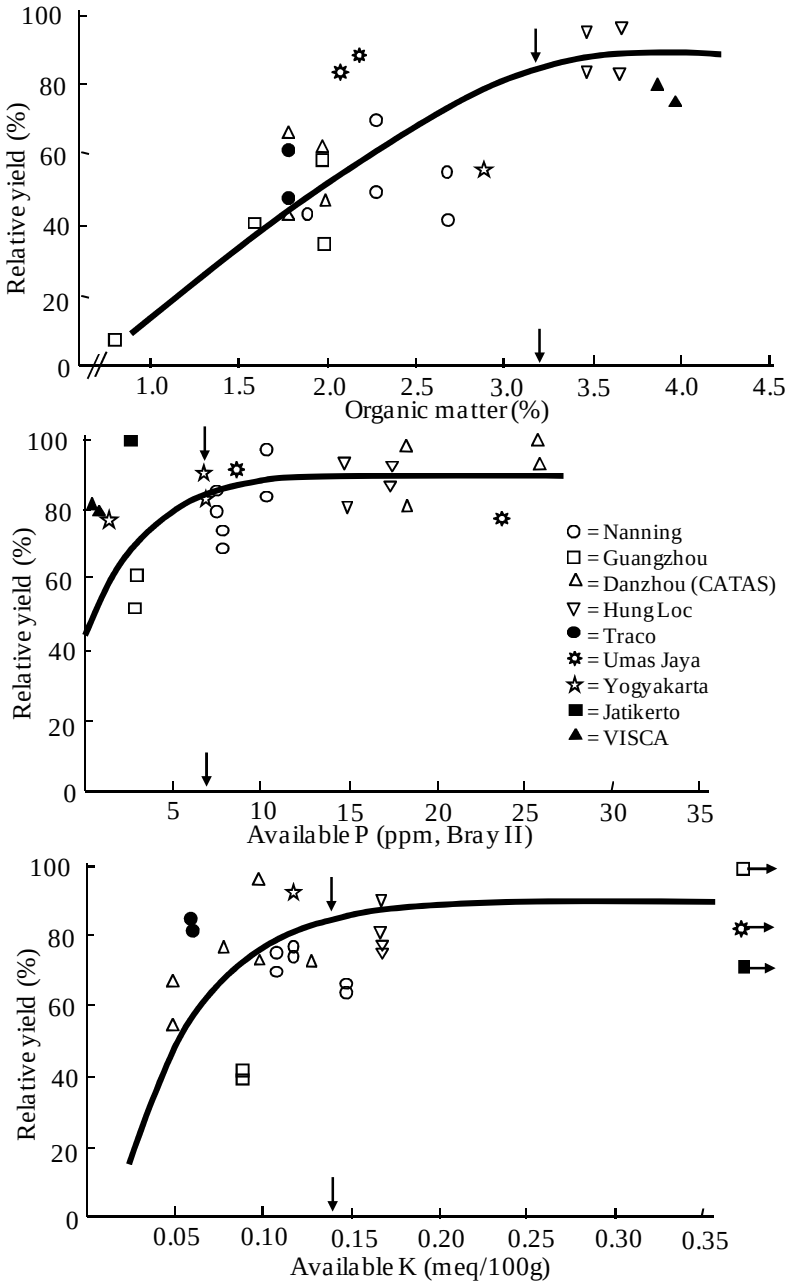

Figure 2. Relation between the relative yield of cassava (i.e. the yield without the nutrient as a percent of the highest yield with the nutrient) and the OM, available P and exchangeable K contents of the soil in nine long-term NPK trials conducted in Asia from 1993-1996. ***Source:*** *Howeler, 1998.*

Table 3. Critical levels[1] of nutrients for cassava and other crops according to various methods of soil analysis, as reported in the literature.

Soil parameter	Method[3]	Crop	Critical level	Source
Organic matter (%)	Walkley and Black	Cassava	3.1	Howeler, 1998
P (ppm)	Bray-I	Cassava	7	Howeler, 1978
		Cassava	8	Kang *et al*., 1980
		Cassava	4.2[2]	Cadavid, 1988
		Cassava	7	Howeler, 1989
		Maize	14	Kang *et al*., 1980
		Soybean	15	Kang *et al*, 1980
	Bray II	Cassava	8	CIAT, 1982
		Cassava	4	Howeler, 1985a
		Cassava	6	CIAT, 1985a
		Cassava	5.8[2]	Cadavid, 1988
		Cassava	10	Howeler, 1989
		Cassava	10	Hagens & Sittibusaya, 1990
		Cassava	4	Howeler & Cadavid, 1990
		Cassava	4.5	Howeler, 1995
		Cassava	7	Howeler , 1998
		Common bean[4]	10-15	Howeler & Medina, 1978
	Olsen-EDTA	Cassava	3	Zaag van der, 1979
		Cassava	7.5[2]	Cadavid, 1988
		Cassava	8	Howeler, 1989
	North Carolina	Cassava	5.0[2]	Cadavid, 1988
		Cassava	9	Howeler, 1989
		Common beans	18	Goepfert, 1972
K (meq/100 g)	NH_4-acetate	Cassava	0.09-0.15	Obigbesan, 1977
		Cassava	<0.15	Kang, 1984
		Cassava	<0.15	Kang & Okeke, 1984
		Cassava	0.18	Howeler, 1985b
		Cassava	0.175[2]	Cadavid, 1988
		Cassava	0.15	Howeler, 1989
		Cassava	0.18	Howeler & Cadavid, 1990
		Cassava	0.08-0.10	Hagens & Sittibusaya, 1990
		Rice	0.21	Jones *et al*., 1982
		Potatoes	0.20-1.00	Roberts & McDole, 1985
		Sugarcane	0.16-0.51	Orlando Filho, 1985
	Bray II	Cassava	0.15	CIAT, 1985a
		Cassava	0.17	Howeler, 1985b
		Cassava	0.16	CIAT, 1988b
		Cassava	0.175[2]	Cadavid, 1988

Soil parameter	Method	Crop	Critical level	Source
	Bray II	Cassava	0.17	Howeler & Cadavid, 1990
		Cassava	0.12	Howeler, 1995
		Cassava	0.14	Howeler, 1998
	North Carolina	Cassava	0.15	Howeler, 1989
Ca (meq/100 g)	NH_4-acetate	Cassava	0.25	CIAT, 1979
		Common beans	4.5	Howeler & Medina, 1978
Mg (meq/100 g)	NH_4-acetate	Cassava	<0.20	Kang, 1984
pH	1:1 in water	Cassava	4.6 and 7.8	CIAT, 1977, 1979
		Common beans	4.9	Abruña *et al.*, 1974
Al-saturation (%)	KCl	Cassava	80	CIAT, 1979
		Common beans	10-20	Abruña *et al.*, 1974

[1] Critical level defined as 95% of maximum yield
[2] Critical level defined as 90% of maximum yield
[3] Methods: Bray I = 0.025 N HCl+0.03 N NH_4F
Bray II = 0.10 N HCl+0.03 N NH_4F
Olsen-EDTA = 0.5 N $NaHCO_3$+0.01N Na-EDTA
North Carolina = 0.05 N HCl+0.025 N H_2SO_4
NH_4-acetate = 1N NH_4-acetate at pH 7

The critical levels for exchangeable K for cassava (0.08-0.18 meq K/100 g) **(Table 3)** are also lower than for most other crops (0.16-0.51 meq K/100 g), indicating that despite the crop's relatively high K requirement, it will still grow well on soils with only intermediate levels of K.

As mentioned above, there is seldom a good relationship between the relative response to N and the soil OM content (Howeler, 1995). Using data from 56 NPK trials conducted in Brazil from 1950-1983 (Gomes, 1998), the critical level determined for OM was only 1.3%, considerably lower than the 3.1% determined in Asia (Howeler, 1998).

Using data from 20 NPK cassava trials conducted in Colombia to compare different methods of extracting available P, Cadavid (1988) reported the highest correlation between relative cassava yields and available soil P using Bray I, followed by Bray II, North Carolina and Olsen-EDTA extracting agents. For determining exchangeable K, Cadavid (1988) found no significant difference between the use of Bray II and NH_4-acetate; both resulted in a critical level of 0.175 meq K/100 g.

3. Plant Tissue Analysis

Analysis of plant tissue indicates the actual nutritional status of the plants at the time of sampling. The total amount of each nutrient is determined, resulting in data that are fairly similar among different laboratories. These analyses are particularly useful for diagnosing N and secondary or micronutrient deficiencies.

Given that nutrient concentrations vary among different plant tissues as well as in different parts of the plant **(Table 4)**, it is imperative to use a specific "indicator" tissue, the nutrient concentration of which is best related to plant growth or yield. For cassava, the best "indicator" tissue was found to be the blade of the youngest fully expanded leaf (YFEL), i.e. normally about the 4^{th}-5^{th} leaf from the top. Leaf petioles should never be mixed with the leaf blades and analyzed together, as nutrient concentrations are quite different in these two tissues. Nutrient concentrations also change during the growth cycle, depending on the rate of plant growth **(Figure 3)** (Howeler and Cadavid, 1983; CIAT, 1985a,b). Since the concentrations of most nutrients tend to stabilize when cassava plants are 3-4 months old, leaf samples should be taken at about 3-4 months after planting (MAP). However, they should not be taken during periods of severe drought or low temperature when plant growth has slowed down. In that case, leaf samples can be taken 2-3 months after normal growth has resumed.

About 20 leaf blades (without petioles) are collected from a plot or uniform area in the field and combined into one sample (Howeler, 1983). If leaves are dusty or have received chemical sprays, they should be washed gently and rinsed in distilled or deionized water. To prevent continued respiration with consequent loss of DM, leaves should be dried as soon as possible at 60-80^{o}C for 24-48 hours. If no oven is available, leaves should be dried as quickly as possible in the sun, preferably in a hot, but well-ventilated area, and away from dust. After drying, samples are finely ground in a lab mill. For Cu analysis samples should be passed through a stainless steel sieve. For Fe analysis the dry leaves should be ground with an agate mortar and pestle. Plant tissue samples are normally collected in paper bags to facilitate drying, but for analysis of B, plastic bags should be used. Once ground and sieved, samples are stored in plastic vials until analysis

To diagnose nutritional problems, the results are compared with the nutrient ranges corresponding to the various nutritional states of the plant **(Table 5)**, or with critical levels reported in the literature **(Table 6)**. Although the numbers may vary somewhat, depending on the varieties, soil and climatic conditions (Howeler, 1983), the data in these tables can be used as a general guide for interpreting plant tissue analyses.

4. Greenhouse and Field Experiments

If analysis of soil or plant tissue is not possible, one can also diagnose nutritional problems by planting cassava in pot experiments using the soil in question, or directly in the field. To diagnose nutrient deficiencies in a particular soil in either pot or field experiments, it is recommended to use the "missing element" technique, where all nutrients are applied to all treatments at rates that are expected to be non-limiting, while one nutrient is missing in each treatment (i.e. -N, -P, -K etc.). Treatments with the poorest growth or yield indicate the element that is most deficient.

For pot experiments it is recommended not to sterilize or fumigate the soil, in order not to kill the native mycorrhizae. Rooted plant shoots rather than stakes should be used as the stakes have high nutrient reserves and their use would therefore delay responses to nutrient additions. In pot experiments cassava plants are generally harvested at 3-4 MAP, and dry weights of top growth are used as indicators of nutrient response.

Table 4. Nutrient concentration in various plant parts of fertilized and unfertilized cassava, cv. M Ven 77, at 3-4 MAP in Carimagua, Colombia.

	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
	(%)						(ppm)				
Unfertilized											
Leaf blades											
Upper	4.57	0.34	1.29	0.68	0.25	0.29	198	128	49	9.9	26
Middle	3.66	0.25	1.18	1.08	0.27	0.25	267	185	66	8.7	37
Lower	3.31	0.21	1.09	1.48	0.25	0.25	335	191	89	7.6	42
Fallen[1]	2.31	0.13	0.50	1.69	0.25	0.22	4850	209	121	9.4	39
Petioles											
Upper	1.50	0.17	1.60	1.32	0.37	0.10	79	172	40	4.4	16
Middle	0.70	0.10	1.32	2.20	0.43	0.10	76	304	72	2.9	15
Lower	0.63	0.09	1.35	2.69	0.45	0.13	92	361	110	2.8	15
Fallen	0.54	0.05	0.54	3.52	0.41	0.13	271	429	94	2.5	18
Stems											
Upper	1.64	0.20	1.22	1.53	0.32	0.19	133	115	36	9.7	14
Middle	1.03	0.18	0.87	1.45	0.30	0.16	74	103	39	8.9	13
Lower	0.78	0.21	0.81	1.19	0.32	0.16	184	95	54	7.9	10
Roots											
Rootlets[1]	1.52	0.15	1.02	0.77	0.38	0.16	5985	191	165	-	10
Thick roots	0.42	0.10	0.71	0.13	0.06	0.05	127	10	16	3.0	4
Fertilized											
Leaf blades											
Upper	5.19	0.38	1.61	0.76	0.28	0.30	298	177	47	10.6	26
Middle	4.00	0.28	1.36	1.08	0.27	0.26	430	207	63	9.6	30
Lower	3.55	0.24	1.30	1.40	0.22	0.23	402	220	77	8.5	37
Fallen[1]	1.11	0.14	0.54	1.88	0.23	0.19	3333	247	120	8.9	38
Petioles											
Upper	1.49	0.17	2.18	1.58	0.36	0.10	87	238	33	4.9	17
Middle	0.84	0.09	1.84	2.58	0.41	0.07	88	359	49	3.0	14
Lower	0.78	0.09	1.69	3.54	0.42	0.07	95	417	70	3.2	15
Fallen	0.69	0.06	0.82	3.74	0.20	0.08	294	471	155	3.1	17
Stems											
Upper	2.13	0.23	2.09	2.09	0.47	0.14	94	140	37	9.8	14
Middle	1.57	0.21	1.26	1.30	0.26	0.11	110	120	46	10.8	12
Lower	1.37	0.28	1.14	1.31	0.23	0.09	210	99	36	10.0	10
Roots											
Rootlets[1]	1.71	0.19	1.03	0.71	0.33	0.20	3780	368	136	-	10
Thick roots	0.88	0.14	1.05	0.16	0.06	0.05	127	15	15	3.9	4

[1] Fallen leaves and rootlets were probably contaminated with micronutrients from the soil.

Source*: *Howeler, 1985a.

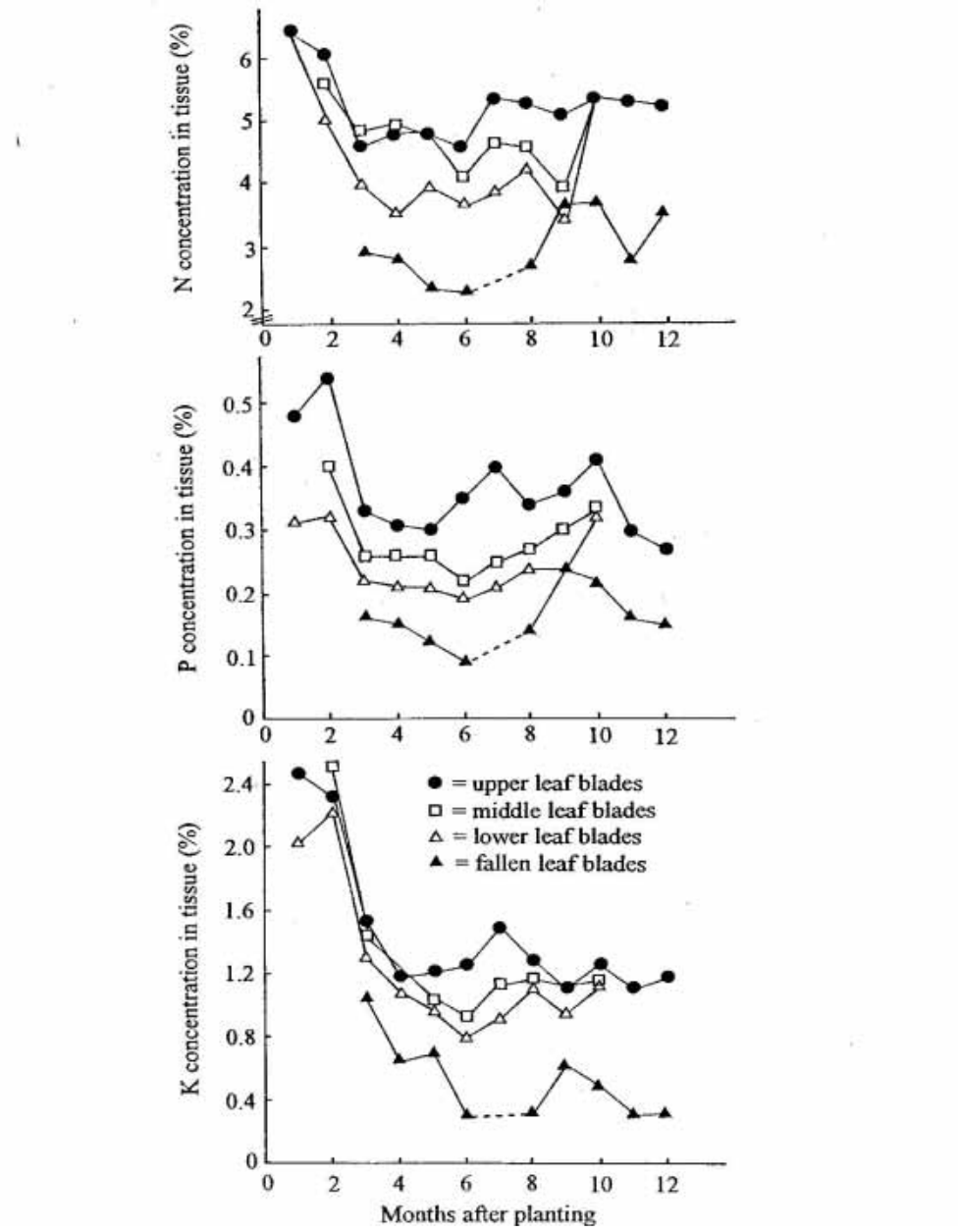

Figure 3. Concentration of N, P and K in leaf blades from the upper, middle and lower part of the plant, as well as from fallen leaves of fertilized cassava cv. M Col 22 during a 12-month growth cycle in Quilichao, Colombia.
Source: *CIAT, 1985a.*

Table 5. Nutrient concentrations in YFEL blades of cassava at 3-4 MAP, corresponding to various nutritional states of the plants; data are averages of various greenhouse and field trials.

	Nutritional states[1)]					
Nutrient	Very deficient	Deficient	Low	Sufficient	High	Toxic
N (%)	<4.0	4.1-4.8	4.8-5.1	5.1-5.8	>5.8	-[2)]
P (%)	<0.25	0.25-0.36	0.36-0.38	0.38-0.50	>0.50	-
K (%)	<0.85	0.85-1.26	1.26-1.42	1.42-1.88	1.88-2.40	>2.40
Ca (%)	<0.25	0.25-0.41	0.41-0.50	0.50-0.72	0.72-0.88	>0.88
Mg (%)	<0.15	0.15-0.22	0.22-0.24	0.24-0.29	>0.29	-
S (%)	<0.20	0.20-0.27	0.27-0.30	0.30-0.36	>0.36	-
B (ppm)	<7	7-15	15-18	18-28	28-64	>64
Cu (ppm)	<1.5	1.5-4.8	4.8-6.0	6-10	10-15	>15
Fe (ppm)	<100	100-110	110-120	120-140	140-200	>200
Mn (ppm)	<30	30-40	40-50	50-150	150-250	>250
Zn (ppm)	<25	25-32	32-35	35-57	57-120	>120

[1)] Very deficient = <40% maximum yield
Deficient = 40-80% maximum yield
Low = 80-90% maximum yield
Sufficient = 90-100% maximum yield
High = 100-90% maximum yield
Toxic = <90% maximum yield

[2)] - = no data available

Source: *Howeler, 1996a, b.*

Table 6. Critical nutrient concentrations for deficiencies and toxicities in cassava plant tissue, as reported in the literature.

Element	Method	Plant tissue	Critical level[1]	Source
N deficiency	Field	YFEL blades	5.1%	Fox *et al.*, 1975
	Field	YFEL blades	5.7%	Howeler, 1978
	Field	YFEL blades	4.6%	Howeler, 1995
	Field	YFEL blades	5.7%	Howeler, 1998
	Nutrient solution	Shoots	4.2%	Forno, 1977
P deficiency	Field	YFEL blades	0.41%	CIAT, 1985a
	Field	YFEL blades	0.33-0.35%	Nair *et al*., 1988
	Nutrient solution	Shoots	0.47-0.66%	Jintakanon *et al*., 1982
K deficiency	Nutrient solution	YFEL blades	1.1%	Spear *et al*., 1978a
	Field	YFEL blades	1.2%	Howeler, 1978
	Field	YFEL blades	1.4%	CIAT 1982
	Field	YFEL blades	1.5%	CIAT, 1982
	Field	YFEL blades	<1.1%	Kang, 1984
	Field	YFEL blades	1.5%	CIAT, 1985a
	Field	YFEL blades	1.7%	Howeler, 1995
	Field	YFEL blades	1.9%	Nayar *et al*., 1995
	Field	YFEL blades	1.9%	Howeler, 1998
	Nutrient solution	Petioles	0.8%	Spear *et al*., 1978a
	Field	Petioles	2.5%	Howeler, 1978
	Nutrient solution	Stems	0.6%	Spear *et al*., 1978a
	Nutrient solution	Shoots and roots	0.8%	Spear *et al*., 1978a
Ca deficiency	Nutrient solution	YFEL blades	0.46%	CIAT, 1985a
	Field	YFEL blades	0.60-0.64%	CIAT, 1985a
	Nutrient solution	Shoots	0.4%	Forno, 1977
Mg deficiency	Nutrient solution	YFEL blades	0.29%	Edwards and Asher, 1979
	Field	YFEL blades	<0.33%	Kang, 1984
	Field	YFEL blades	0.29%	Howeler, 1985a
	Nutrient solution	YFEL blades	0.24%	CIAT, 1985a
	Nutrient solution	Shoots	0.26%	Edwards and Asher, 1979
S deficiency	Field	YFEL blades	0.32%	Howeler, 1978
	Nutrient solution	YFEL blades	0.27%	CIAT, 1982
	Field	YFEL blades	0.27-0.33%	Howeler, unpublished
Zn deficiency	Field	YFEL blades	37-51ppm	CIAT, 1978
	Nutrient solution	YFEL blades	43-60 ppm	Edwards and Asher, 1979
	Nutrient solution	YFEL blades	30 ppm	Howeler *et al*., 1982c
	Field	YFEL blades	33 ppm	CIAT, 1985a
Zn toxicity	Nutrient solution	YFEL blades	120 ppm	Howeler *et al*., 1982c

Element	Method	Plant tissue	Critical level[1]	Source
B deficiency	Nutrient solution	YFEL blades	35 ppm	Howeler *et al*., 1982c
	Nutrient solution	Shoots	17 ppm	Forno, 1977
B toxicity	Nutrient solution	YFEL blades	100 ppm	Howeler *et al*., 1982c
	Nutrient solution	Shoot	140 ppm	Forno, 1977
Cu deficiency	Nutrient solution	YFEL blades	6 ppm	Howeler *et al*., 1982c
Cu toxicity	Nutrient solution	YFEL blades	15 ppm	Howeler *et al*., 1982c
Mn deficiency	Nutrient solution	YFEL blades	50 ppm	Howeler *et al*., 1982c
	Nutrient solution	Shoots	100-120 ppm	Edwards and Asher, 1979
Mn toxicity	Nutrient solution	YFEL blades	250 ppm	Howeler *et al*., 1982c
	Nutrient solution	Shoots	250-1,450 ppm	Edwards and Asher, 1979
Al toxicity	Nutrient solution	Shoots	70->97 ppm	Gunatilaka, 1977
	Nutrient solution	Roots	2,000-14,000 ppm	Gunatilaka, 1977

[1)] Range corresponds to values obtained in different varieties.

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CHAPTER 13

CONDUCTING CASSAVA EXPERIMENTS IN THE GREENHOUSE AND FIELD[1]

Reinhardt Howeler[2]

INTRODUCTION

The nutritional requirements of plants can be studied in nutrient solutions, sand cultures and pot experiments with soil in the greenhouse, as well as by experiments in the field. In all these experiments the response of the plants to various levels or concentrations of some element (or elements) are determined. In addition, one can determine the relation between the concentration of the element in the soil or in a certain indicator tissue in the plant in order to define the external or internal requirement, respectively, of that element.

In this chapter some basic techniques for nutritional studies in the greenhouse or field are discussed, as well as some non-conventional techniques, such as the "programmed nutrient addition" and the "flowing nutrient solution" techniques used in the greenhouse, and systematic designs sometimes used in field experiments.

The "nutritional requirement" of a crop can be defined in several ways:

1. The amount of nutrients that the plant absorbs from the soil during its growth cycle. In general, the rate of nutrient absorption depends on the rate of growth of the plant, which in turn is a function of its genetic potential, the fertility of the soil, the climate etc. Several examples in Chapter 14 show how the accumulation of dry matter (DM), N, P, K, Ca, Mg etc in cassava depends on many of these factors. This type of "nutrient requirement" can be determined by frequent sampling of various plant tissues during the growth cycle, to determine the production of dry matter and the concentration of nutrients in the various plant parts.
2. The amount of nutrients to be applied to the soil to obtain "optimum" growth and production. This is generally defined as 95% of maximum production. It is considered to correspond with the "agronomic optimum", which is not necessarily the same as the "economic optimum". This definition of "nutritional requirement" is similar to the "fertilizer requirement" and will vary from one soil to the other, depending on the original fertility of the soil and the climatic conditions.
3. The concentration of nutrients in the soil or in the plant corresponding to optimum production. This definition of "nutritional requirement" is the same as the so-called "critical level of deficiency" in the soil or in the plant, i.e. the concentration of the nutrient below which the plant will respond to the application of the nutrient and above which no response is expected. Similarly, the excessive availability of an element is determined by the "critical level of toxicity", which is the concentration above which the growth will decrease due to the excessive uptake of the element. The critical level in some indicator tissue of the plant is also called the "internal nutrient requirement", while the critical level in the growth medium, like the soil, the soil solution or the

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nutrient solution, is called the "external nutrient requirement". The critical level is considered a rather constant characteristic of the species or of the variety, and more or less independent of the soil and climate. As such, critical levels can be determined in nutrient solution cultures, in pots with soil in the greenhouse, as well as directly in the field.

Although the critical level is considered a rather constant characteristic of the species, this level can still vary somewhat between varieties of the same species. Moreover, it varies between different tissues of the same plant, and with the age of the tissue. It is also affected to some extent by the presence or absence of other nutrients and by the environmental conditions like temperature, rainfall etc. As an example, **Table 1** shows the N, P and K concentrations in upper leaves of the same cassava variety grown at the same time in three sites with similar soils but at different elevations above sea level. The plants growing at the lowest elevation produced the greatest growth but had the lowest concentrations of N, P and K in the leaves as compared with those plants growing at higher elevations and at a much lower temperature. Thus, in the interpretation of the analytical data to determine the nutritional status of the plants, one has to take into consideration the rate of growth of the plants and its physiological state, which can vary according to the environmental conditions.

In this chapter we will only consider the techniques to determine the nutritional requirements according to the second and third definition, i.e. the critical levels and the fertilizer requirements, respectively.

Table 1. Effect of temperature on the growth and the concentration of nutrients in the upper leaf blades of cassava, MCol 113, at four months after planting [1)].

	← Location of planting →		
	Quilichao	Mondomito	Agua Blanca
Elevation above sea level (m)	990	1450	1520
Medium annual temperature (ºC)	25	21	19
pH of the soil	4.2	4.1	4.4
Organic matter (%)	7.5	5.6	6.1
Available-P (Bray II) (ppm)	1.8	1.6	0.8
Exchangeable K (meq/100g)	0.18	0.14	0.16
Cassava plant height (cm)	80.2	54.3	55.7
%N in upper leaf blades	4.36	5.55	5.57
%P in upper leaf blades	0.24	0.35	0.38
%K in upper leaf blades	1.32	1.62	1.68

[1)] Planted at the same time in three different locations in Colombia. Data are the average of 12 NPK treatments

Determination of Critical Levels in the Soil

The critical levels in the soil can be determined by conducting fertilizer trials in the field or in pots with soil in the greenhouse. In both cases, the response of the plants to the application of various levels of the nutrient is determined, and growth or yield of the plants is then related to the concentrations of the nutrient in the soil. This is done by drawing a

curve which best describes the relation between yield and the particular nutrient concentration in the soil. Normally, this relationship is curvilinear with a defined maximum. The "critical level" is normally defined as the concentration of the nutrient in the soil that corresponds to 95% of the maximum yield **(Figure 1)**. If the curve does not reach a maximum, this means that the highest level of application was not high enough for the plants to reach their maximum production. In that case, it will not be possible to determine the critical level.

The determination of critical levels in the field has the advantage of using real yield data of grain or roots instead of that of dry matter production, as is normally determined in greenhouse experiments. However, the latter have the advantage that it is possible to determine in one location under identical environmental conditions the critical levels in a range of soils from various locations and with different characteristics.

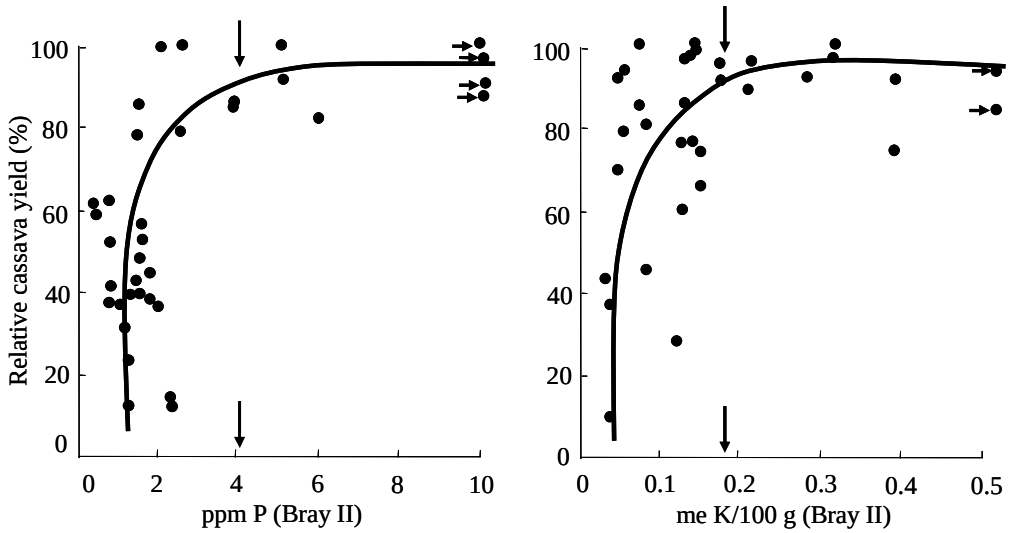


Figure 1. Relation between the relative cassava yield of P or K check plots and the available P (left) of exchangeable K (right) content of the soil in 24 NPK trials in Colombia. Vertical arrows indicate the critical levels corresponding to 95% of maximum yields

Determination of Critical Levels in the Plant

To determine the critical levels in the plant, the yields are related to the concentration of the nutrient in certain organs of the plant as obtained from experiments conducted in the field, or from experiments using pots with soil or nutrient solutions in the greenhouse. It is very important to define the indicator tissue to be used and the growth status of the plant, because the nutrient levels will vary a lot between different parts of the plant and will change during the growth cycle (see Chapter 12). Howeler (1983) has summarized results in the literature about the growth stage and the plant part to be sampled for various tropical crops. He concluded that for cassava the best indicator tissue is the blade of the youngest fully-expanded leaf (YFEL), i.e the 4^{th} or 5^{th} leaf at the top of the plant, at about 3-4 months after planting. The critical level of the nutrient is again that

concentration in the indicator tissue corresponding with 95% of maximum yield **(Figure 2).** In order to determine whether a plant is well nourished, you have to sample and analyze the indicator tissue at the right stage of the growth cycle (but never during periods of drought or low temperatures when growth is very slow). If the concentration found in the tissue is below the critical level, it is expected that the crop will respond positively to the application of the nutrient in question; if it is above the critical level one would not expect a response to the application.

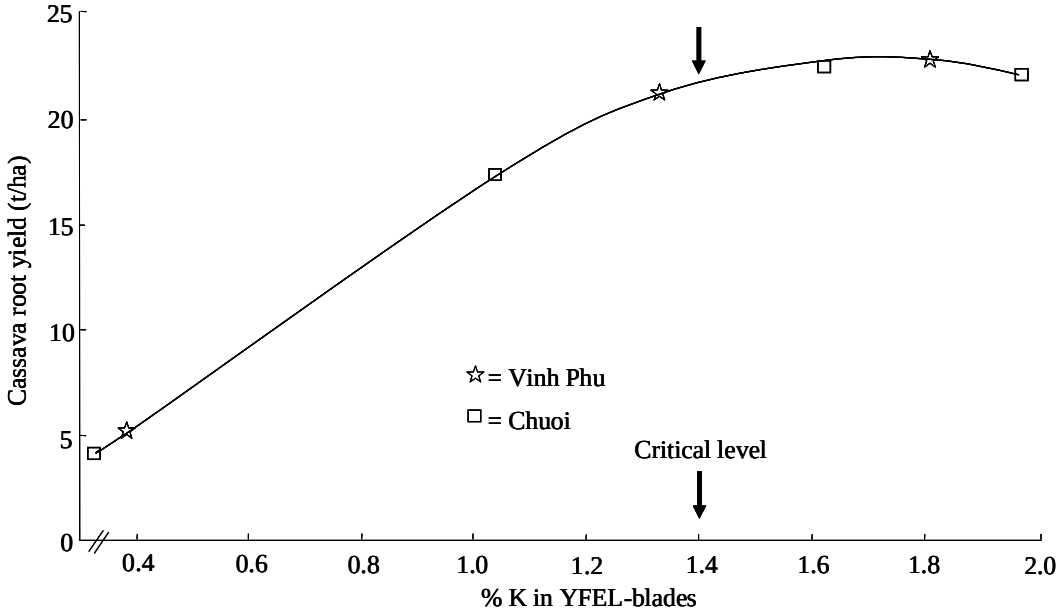


Figure 2. Relation between the root yields of two cassava varieties and the K concentration in the youngest fully-expanded leaf blades at three months after planting at Thai Nguyen University in Thai Nguyen, north Vietnam.

GREENHOUSE EXPERIMENTS

Pot Experiments with Soil

Pot experiments with soil are useful to determine quickly which nutrients are most important for obtaining optimum growth of plants in any particular soil. This type of trials can also be used to determine the critical level of nutrients in the indicator tissue of plants by applying different rates of a nutrient to the soil and plotting the concentration of the nutrient against the total DM produced at a certain age of the plant. In case of cassava this is best done at about three months after planting. Growth of cassava beyond that stage is often restricted by the small volume of soil in the pot available for normal root development.

The soil used in pot experiments is usually sun-dried before weighing out a constant weight for each pot. Normally, the soil should NOT be sterilized as that would kill all the micro-organisms in the soil, including the VA-mycorrhiza, which are very

important for the uptake by cassava of P and some micro-nutrients. It is also not advisable to use normal cassava cuttings in pot experiments, as these will contain a rather large reserve of nutrients, which would delay any response to nutrient applications. Instead, in both pot experiments with soil or sand culture, and in nutrient solution trials it is better to use small rooted cassava plantlets, which have relatively little nutrient reserves and thus will respond quickly to the application of any nutrient that is in short supply in the growth medium. These rooted plantlets can easily be produced in beds filled with a low fertility soil and planted densely with small (5-10 cm long) cuttings of the cassava test variety. Once these cuttings have sprouted and the shoots are about 15 cm long, these are cut off with a sharp razor blade and the bottom end of the shoots are placed in small bottles filled with cool boiled water. The water needs to be replaced regularly to prevent accumulation of latex exuding from the cut shoots. After several weeks these shoots will have developed small roots and are then ready for transplanting in the pots with soil or nutrient solutions. It is recommended to separate the plantlets into three or four groups according to the size or vigor of the plantlets, and to use the plants from each group for planting in each of the replications used in the experiment; this is done to reduce the variability within each replication.

Nutrient Solution Experiments Using Fixed Nutrient Concentrations

This is the simplest and most commonly used method in nutrient solution experiments. Nutrient solutions are prepared in which the concentration of one nutrient varies among treatments, while all the other nutrients are supplied at a fixed concentration deemed optimum for normal plant growth. An example of the preparation of nutrient solutions used for cassava is shown in **Table 2**. To avoid marked changes in the nutrient concentrations of the solution due to the absorption of nutrients by the plants, rather high concentrations are used (often several orders of magnitude higher than found in the soil solution). In addition, the solutions need to be changed rather frequently. The disadvantage of this method is that the solutions are often initially too concentrated when the plants are small and growing slowly, while during the period of maximum growth rates the absorption of nutrients is so high that the concentrations decrease rapidly with time. These changes in the concentration can result in the determination of critical levels which tend to be too low (Spear *et al.*, 1979).

Nutrient Solution Experiments Using Programmed Nutrient Additions

This method has been proposed by Asher and Cowie (1970) and involves the programming of nutrient additions according to the rate of growth of the plants, i.e. the additions are initially little, but as the plants grow the concentrations in the solutions are increased. To calculate the concentrations of the mother solutions that are added every week one has to know the expected rate of growth during the following week and the average concentration of each nutrient in the plant.

Table 2. Preparation of nutrient solutions with fixed concentrations for cassava.

Element	Concentration in the nutrient solution (ppm)	ml of mother solution per 45 liters of nutrient solution	Source	g/2.5 liters of mother solution
N	80	50	$Ca(NO_3)_2.4H_2O$	1518.11
P	4	40	$NaH_2PO_4.2H_2O$	56.67
K	40	25	K_2SO_4	401.13
Ca	57.25			
Mg	20	25	$MgSO_4.7H_2O$	912.89
S	42.97			
Mn	0.1		$MnSO_4.4H_2O$	1.83
Mo	0.05		$Na_2MoO_4.2H_2O$	0.57
B	0.2	25	H_3BO_3 *	5.15
Zn	0.1		$ZnSO_4.7H_2O$	1.98
Cu	0.05		$CuSO_4.5H_2O$	0.88
Fe	2		$FeCl_3.6H_2O$ in citric acid	43.56

*The minor elements are dissolved separately and then mixed with 125 ml concentrated H_2SO_4, after which the volume is completed to 2.5 liters with double deionized water.

Figure 3 shows an example of the growth rate of cassava in nutrient solution. The growth of the plants generally follows an exponential curve according to the formula:

$$W = e^{xt}$$

where W = dry weight of the plant
x = the average rate of growth
t = time in days

The change in dry weight of the plant during a certain period of growth will be:

$$\ln W_2 - \ln W_1 = x(t_2 - t_1)$$

where W_2 = dry weight at t_2
W_1 = dry weight at t_1

The rate of growth is not always constant but tends to decrease over time, as can be seen in **Figure 3**.

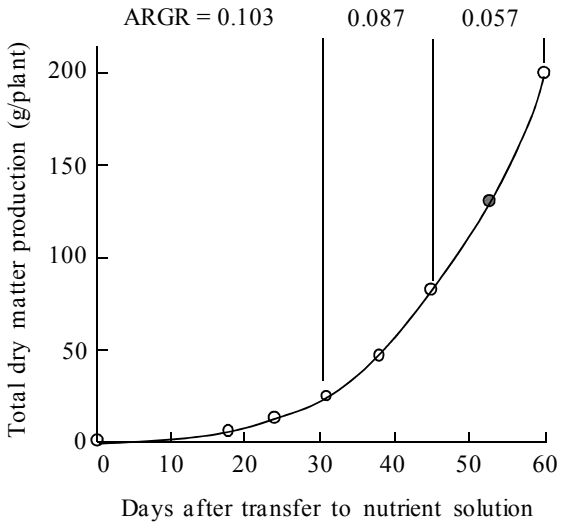


Figure 3. Growth rate of cassava growing in nutrient solution. Above the curve is shown the calculated average relative growth rate (ARGR).

In any case, one can estimate the growth rate during the following week from the measured growth rate during the preceding week, and then calculate the approximate expected dry weight at the end of the following week. Knowing the approximate average concentration of each nutrient in the plant enables you to calculate the absorption of nutrients during each week. Those amounts are added to the nutrient solution at the beginning of each week. One must assume that all nutrients added to the solution previously had been absorbed. **Table 3** is an example of the calculations in the case of cassava. With this technique the nutrients are added at the rate that the plants need them, resulting in vigorous growth of well-nourished plants. Moreover, one can apply different levels of each element to study the response of the plants, and to determine the critical levels of those elements in the plants.

Flowing Nutrient Solution Experiments

This method, which requires much more sophisticated equipment than the previous two methods, has as its objective to maintain the concentration of some element constant during the whole growth cycle. This is achieved by using very large volumes of nutrient solution which are pumped constantly across all the pots having the same treatment. The nutrient solutions are analyzed daily and nutrients are added in the same amount as that which the plants had absorbed during the previous day. In addition, a solution containing the element under study is added continuously by drops at the rate of its expected absorption.

This way it is possible to maintain the concentration constant and at a very low level, which corresponds with that normally found in the soil solution. Since the soil is a buffered system, the soil particles constantly supply nutrients to the soil solution at the rate that these nutrients are absorbed by the plants. The system of flowing nutrient solutions tries to simulate the buffering capacity of a natural soil, by maintaining the concentrations

constant through continuous nutrient additions. This way it is possible to study the absorption and translocation of nutrients in a medium that approximates that of a natural soil. For more details about this technique, see Asher and Edwards (1978).

Table 3. The rate of growth of cassava and the amount of nutrients in the produced dry matter.

Expected growth in dry matter:

$$W = e^{xt} \longrightarrow x = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

where : W = dry weight (DW)
x = average relative growth rate (ARGR)

Days	Average relative growth rate (ARGR)	DW of plants (g)	Change in DW (g)
0		1.0	
18	0.103	6.38	5.38
24	0.103	11.85	5.47
31	0.103	24.36	12.51
38	0.087	44.79	20.43
45	0.087	82.35	37.56
53	0.057	129.93	47.58
60	0.057	193.64	63.71

Nutrient concentration in the plant:			
5.0% N	0.5% P	2.0% K	1.5% Ca
0.5% Mg	0.65% S	15 ppm Cu	100 ppm Fe
100 ppm Mn	50 ppm Zn	15 ppm B	
For each gram of dry weight produced we have to add:			
50 mg N	5 mg P	20 mg K	15 mg Ca
5 mg Mg	6.5 mg S	15 µg Cu	100 µg Fe
100 µg Mn	50 µg Zn	15 µg B	

FIELD EXPERIMENTS

Many cassava field experiments have been conducted over the past 40 years and a wealth of information has been obtained. Still, many questions remain to be answered as results have not always been conclusive, while some results also need to be confirmed or adapted to particular local conditions. Thus, additional experiments will need to be

conducted, either on-station or on-farm, which are both designed and managed by researchers; or in farmer participatory research (FPR) trials which are designed and managed by farmers with help from researchers. This chapter provides some guidance about how to conduct these experiments and how to calculate the yields of cassava as well as those of intercrops or other associated crops that are planted in the experiment.

Plot Size and Shape and the Need for Border Rows in Cassava Experiments

Cassava is a vegetatively propagated crop and most experiments are planted with about 20 cm long stem cuttings, also called "stakes". Mature plants are quite large and each plant will therefore require considerable space, enough to minimize plant-to-plant competition but to maximize yields. In many cases planting material is limited and should be used as efficiently as possible. Also, the length and thickness of each stake will differ, as well as the maturity of the stem from which it was cut will differ, resulting in large variability between individual plants in the experiment. To reduce the coefficient of variation, experimental plots will need to be relatively large, the plant stand need to be as complete as possible, and plant growth should be as uniform as possible. Unlike rice or maize where each plot may have hundreds of plants, in cassava experiments the plot size may be larger but the number of plants per plot will probably be much smaller. Thus, every plant counts and each plant makes a considerable contribution to the total yield determination

Research to determine the minimum number of harvested plants per plot was conducted at CIAT in the early 1970s (CIAT, 1974). While the results varied between different varieties, the preliminary recommendation was to use a minimum of 25 harvested plants per plot, to use square plots with two border rows (not harvested for yield determination) and six replications when using a randomized complete block design. In practice, however, smaller plots with a minimum of 12-16 harvested plants in the so-called "effective plot" and with only one border row on all sides, and 3-4 replications are often used to reduce costs, to safe planting material and to keep the trial within a reasonable size, say 0.25 ha, with as uniform soil conditions as possible. As with any other crop, the experiment should be laid out in such a way that any existing variation in slope or soil occurs between replications, while the variation within each replication is held to a minimum.

Most cassava fibrous roots are present in the top 20 cm of soil, but some roots can go down as much as one meter depth while others may grow 1-2 meters sideways. Thus, interplant competition does not only occur above-ground by shading, but also by roots underground for the uptake of water and soil nutrients. In the center of a field (or plot) each plant is surrounded by eight neighbors, which all compete with each other for light, water and nutrients. Plants growing in a border row are surrounded by only five neighboring plants and those growing at the plot's corner by only three neighboring plants; these plants are thus subjected to less competition and have a higher yield than those plants in the center of the plot. To calculate the "true" yield of a certain treatment we should only harvest plants fully surrounded by other plants inside the "effective plot" and exclude any plants growing in border rows, as the latter have had less competition. Border row plants are generally taller and have higher yields than those inside the plot, because they receive

more light, and can extend their roots into alleyways or neighboring plots to absorb additional nutrients or water not corresponding to the treatment of their own plot.

It is recommended to use square plots because these have a greater number of plants inside the "effective plot" in relation to the total number of plants in the plot with borders. For instance, using a planting distance of 1.0 x 1.0 m and a square plot of 6 x 6 m or 36 m^2, there will be 4 x 4 = 16 plants in the "effective plot", which is surrounded on all four sides by one border row. The yield will be calculated from the yield of these 16 plants. If we use a long narrow plot of 9 x 4 m or the same 36 m^2 , there will only be 7 x 2 = 14 plants in the "effective plot". Using the square plot we can use 16/36 = 44% of the plants to calculate the yields, while using the rectangular long and narrow plot we can use only 14/36 = 39% of the plants to calculate the yield. For that reason, square or squarish plots give you a better yield estimate than long narrow plots with the same number of plants.

Figure 4 indicates that as the plant population increased from 3,000 (spacing 1.8x1.8 m) to 40,000 (spacing 0.5x0.5 m) plants/ha, the root yield per plant decreased due to increasing interplant competition. Furthermore, border plants in the first row had significantly higher yields, while those in the second border row had slightly higher yields, and those in the third border row had the same yield as plants in the plot's center which had at least three border rows. Thus, to obtain a true estimate of treatment effects on yield, at least one and preferably two border rows should not be included in the "effective plot" that is harvested for yield determination.

It is not necessary to have the same plot or effective plot size throughout the experiment, especially in plant spacing trials. It is important, however, to harvest at least a minimum of 16-25 plants per plot (in the effective plot) to reduce the coefficient of variation. This means that those treatments having closer plant spacing (higher plant density) can be planted in smaller plots, while treatments having a wider spacing may need bigger plots.

Laying Out an Experiment with 90 Degree Corners

Before laying out an experiment, the available area should be carefully observed to see whether there is any consistent variation in slope or soil conditions. If so, the replications should be laid out in such a way that the existing variation occurs between replications and not within each replication. Thus, replications should be laid out perpendicular to the slope or to the soil fertility gradient. Furthermore, plots should be at least 5-10 meters away from trees because trees not only affect plant growth in nearby plots through shading, but tree roots can extend far beyond the shade line and absorb water and nutrients within a 5-10 m radius surrounding the tree, depending on the height and type of tree.

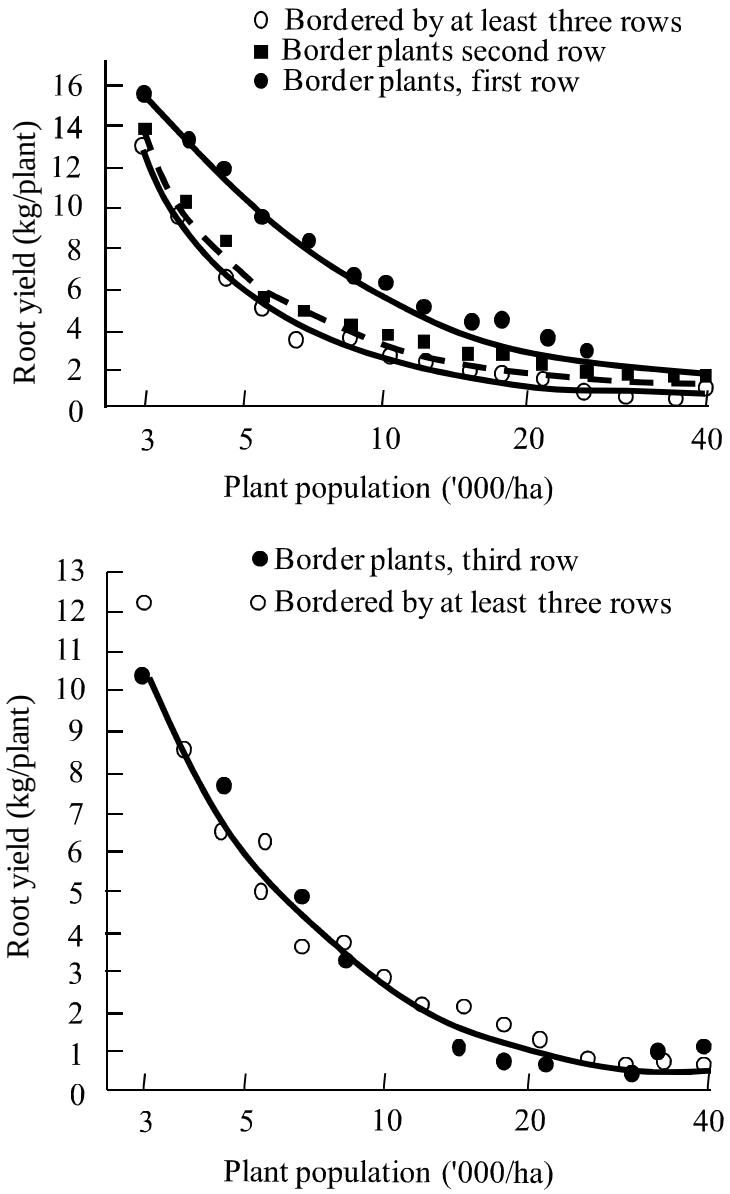


Figure 4. Yield of border plants of cassava, cv. Llanera, at different plant populations.
Source: *CIAT, 1974.*

Once the general shape of the experiment is determined, you stake out a baseline, using stakes and string, corresponding to the longest side of the experiment. At one end, a line perpendicular to the base line is set out by measuring exactly 8 meters along the baseline, 6 meters along the perpendicular line and exactly 10 meters along the diagonal line, as shown in **Figure 5.** Any multiple of a 3:4:5 ratio will make a 90 degree angle.

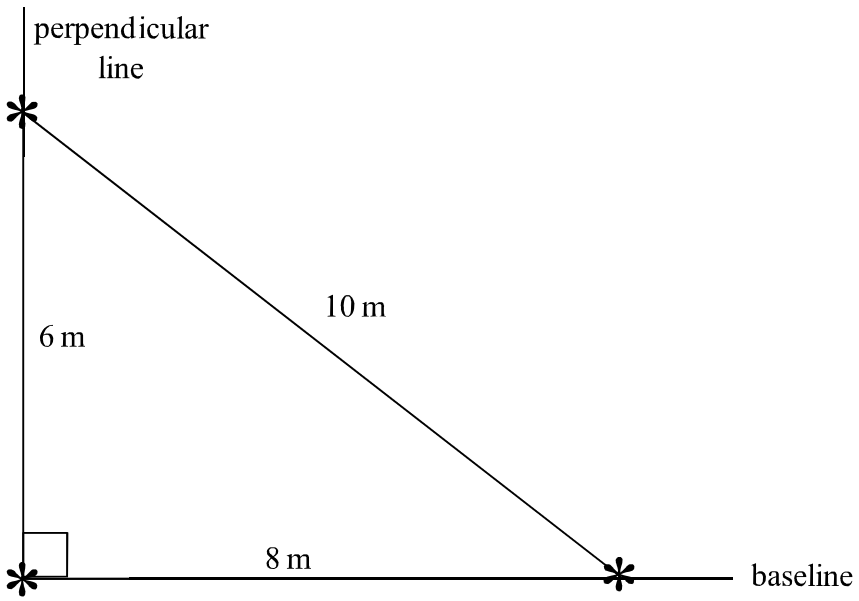


Figure 5. Simple way of setting out a 90 degree corner in field experiments.

The plots are staked out according to the experimental plan along the baseline and the perpendicular line. The two other sides of the experiment are staked out in a similar fashion by setting out another 90 degree angle and making sure that the two long sides and the two short sides of the experiment are indeed of the same lengths, respectively. The plots and replications are staked out along all four external border lines and the stakes are then connected with string to lay out all plots.

Plant Spacing and Lay-out

In any experiment cassava should be planted at a uniform plant spacing, either throughout the whole experiment, or in each treatment in case of a plant spacing trial. To simplify the laying out of experiments, a planting distance of 1.0 x 1.0 m is often used; this also corresponds to the near optimum spacing for most cassava varieties planted in fertile soils; in infertile soils or when cool climates result in slow growth, a closer spacing of 0.8 x 0.8 m or 0.8 x 0.9 m is more appropriate.

The first row of cassava should never be planted on the plot border line as it would be impossible to say to which plot the plants in this row belong. Instead, the first row is generally planted at half the planting distance from the border line and the last row is also planted at half the planting distance from the opposite plot border line; similarly, the first and last plants in each row are planted at half the planting distance from the perpendicular plot border line, as shown in **Figure 6**.

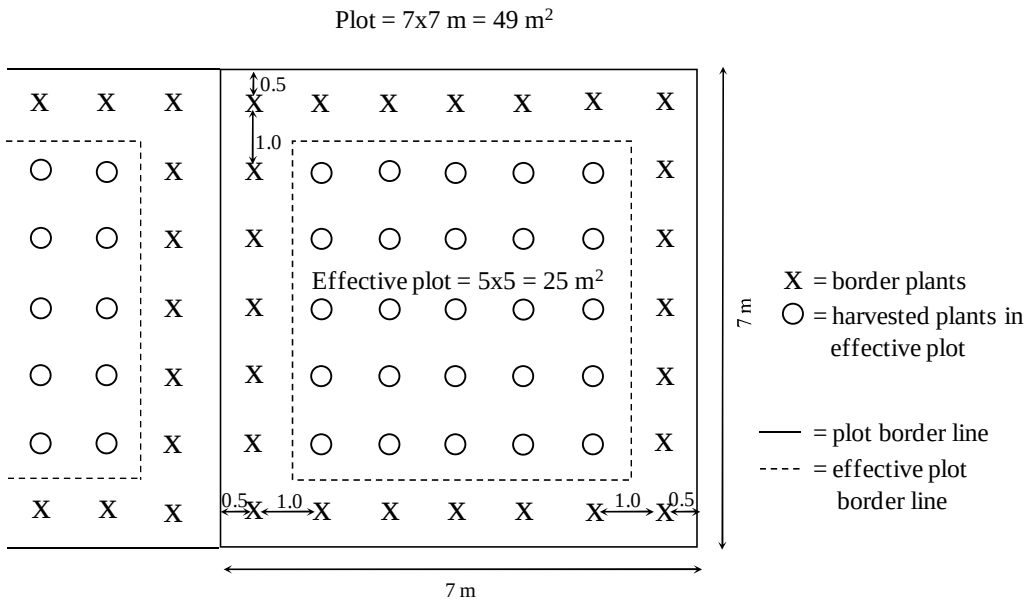


Figure 6. Lay-out of cassava experimental plot with cassava planted at 1.0 x 1.0 m spacing and plot size of 7.0 x 7.0 m.

Since it is important to have as complete a plant stand as possible, especially within the effective plot, it is advisable to plant a few extra stakes in the space between the first row and the plot border line in each plot. These can be used for transplanting to replace those plants that have not germinated at 2-3 weeks after planting (WAP). Alternatively, new stakes of the same variety are used to replant in the empty spaces where the original stakes had not germinated. This "gap filling" should be done as soon as most stakes have germinated, usually at 2-3 WAP, so as to obtain as uniform a plant growth within the plot as possible. If gap filling is done too late the new plants will not be able to compete with their taller neighbors and will never catch up and produce normal yields (**Table 4**).

Common Experimental Designs Used in Cassava Field Experiments

There are many experimental designs that can be used, the most common being the randomized complete block (RCB) design, the split plot, the split-split plot, and the complete or incomplete factorial designs. For varietal evaluations the most common is an RCB design, but in fertilizer trials a split plot, split-split plot or incomplete factorial design is often used. In that case, the main plots often have two or more varieties and the subplots have different fertilizer treatments. In long-term fertilizer trials there is always the danger that previously applied fertilizers are moved across plot borders during land preparation or weeding, especially when using tractors, and thus contaminating the neighboring plots. To reduce this problem, the fertilizer treatments should be in the largest plots, i.e. the main plots, while the different varieties are planted in subplots within the main plots. Similarly, in experiments on land preparation methods using various tractor-mounted implements, these treatments require rather long plots to enable the tractor to move at a constant speed; in that case the land preparation treatments are usually in the main plots, while different varieties can be planted in subplots.

Table 4. Yield of cassava (cv. Golden Yellow) under different periods of replanting missing hills in ViSCA, Leyte, Philippines.

Replanting time of missing hills	Total[1)] root yield (t/ha)	Root yield[2)] of sample plants (kg/plant)
1. Control, 0 missing hill (MH)	20.06 a	1.71
2. 35% MH unreplanted	22.87 a	2.62
3. 35% MH replanted 13 days after planting (DAP)	22.93 a	0.96
4. 35% MH replanted 20 DAP	19.56 a	0.48
5. 35% MH replanted 27 DAP	18.20 a	0.11
6. 40% MH unreplanted	21.09 a	3.68
7. 40% MH replanted 13 DAP	19.78 a	1.03
8. 40% MH replanted 20 DAP	14.98 b	0.54
CV (%)	14.26	28.13

[1)] Mean separation (LSD, 0.05)

[2)] The replanted plants (tr. 3, 4, 5, 7 and 8) or those adjacent to missing hill (tr. 2 and 6) or those with complete borders (tr. 1)

Source: *Villamayor, 1988.*

The Use of Systematic Designs

To study in detail the interaction between two factors, for instance the response to various levels of P and K applied, one can use a systematic design where treatments are not arranged randomly but in a systematic order, from zero to the highest level, one factor in one direction and the other in the perpendicular direction. In case of cassava, each plot may be only 1 m^2 in size and have only one plant. There is no need for border rows between plots because neighboring plots have only slightly different treatments; but two border rows are necessary along the outside borders of the experiment. Thus, an experiment with 12 levels of P by 12 levels of K has 144 treatments, and with four replications and two outside border rows occupies only 28 x 28 = 784 m^2. An example is shown in **Figure 7** in which the levels of P and K are as shown in **Table 5**.

To reduce the effect of plant-to-plant variability, the yield of plant "x" is calculated by moving averages, i.e. the average yield of the plant "x" in the plot and its eight surrounding neighbors. For example, the yield of treatment P2K2 is the average yield of the nine plots, P1K1, P1K2, P1K3, P2K1, P2K2, P2K3, P3K1, P3K2 and P3K3, while the yield of treatment P2K3 is the average yield of P1K2, P1K3, P1K4, P2K2, P2K3, P2K4, P3K2, P3K3 and P3K4. Thus, the effective plot of each treatment consists of nine plants. This method of moving averages can be used because the difference between neighboring treatments is minimal, and in any case it may be assumed that the yield differences of neighbors on opposite sites of plant "x" will cancel out. In this way the yield of each plant is used nine times in the analysis, thus increasing the efficiency of the experiment.

While the size of the experiment is rather small, each plant will need to be harvested and the roots weighed individually; the weight of each plant is recorded separately to be able to calculate the yield of each treatment as the average yield of nine plants. This design does not allow an analysis of variance, but it is a very useful design to demonstrate in the field the response to different levels of nutrients and their interaction. Using this design, the interaction between N and K, P and K, or lime and P have been studied in four types of soil in Colombia (CIAT, 1977, 1978, 1979).

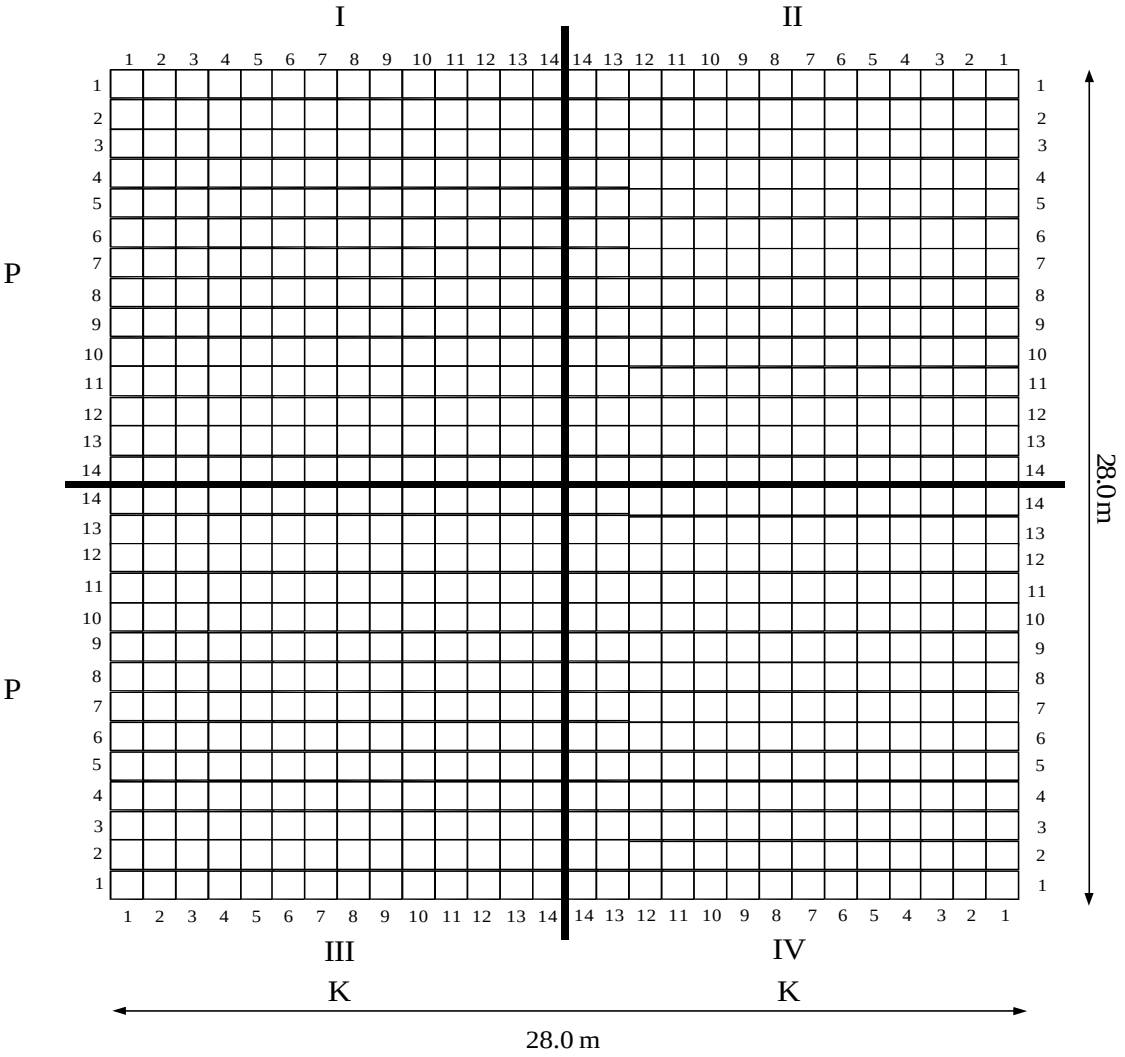


Figure 7. Systematic design to study the interaction between two nutrients (P and K) applied at 14 levels, increasing at constant increments from zero to the highest level. Each plot is 1 m^2 and has one cassava plant; the trial has four replications

Table 5. Example of the levels of P and K used in a Systematic Design to determine the interaction of these two nutrients in a particular soil.

Treatment	P (kg/ha)	K (kg/ha)
1	0	0
2	0	0
3	0	0
4	10	15
5	20	30
6	30	45
7	40	60
8	50	75
9	60	90
10	70	105
11	80	120
12	90	135
13	100	150
14	100	150

The systematic design can also be used to determine the requirements for a certain nutrient of several crops or varieties, by planting strips of each crop or variety across narrow strips with levels of the nutrient increasing systematically and in constant increments **(Figure 8)**. This design can be used to determine quickly and in a small area the nutritional requirements of any crop in a particular soil. If necessary, the results can be confirmed by a subsequent experiment using a more conventional design using fewer levels within the range defined by the systematic design

Application of Fertilizers, Manures and Lime

Fertilizers and soil amendments (like lime or manures) can be divided into two general classes: those that are readily soluble in water and those that need time and good contact with the soil to dissolve or decompose. Most chemical fertilizers, such as urea, single superphosphate (SSP), triple superphosphate (TSP), potassium chloride (KCl), potassium sulfate (K_2SO_4), magnesium sulfate ($MgSO_4$), zinc sulfate ($ZnSO_4$) and various compound NPK fertilizers dissolve rapidly in water. They can be spot- or band-placed at 5-10 cm from the planted stake. These fertilizers will dissolve in the soil solution and the roots will tend to grow towards the fertilizer band. A single hole or short band at 10 cm on one side of the stake or plant is made with a pointed stick or hoe, the fertilizer (or mixture of several fertilizers) is placed in the hole and then covered with soil. Fertilizers should never be left on top of the soil as nutrients may be lost by volatilization or by runoff or erosion, nor should they be in direct contact with the planted stake as this may affect germination. The advantage of spot or band placement is that the fertilizer is concentrated near the cassava plants which will benefit from it, while most weeds will not be able to access the fertilizers.

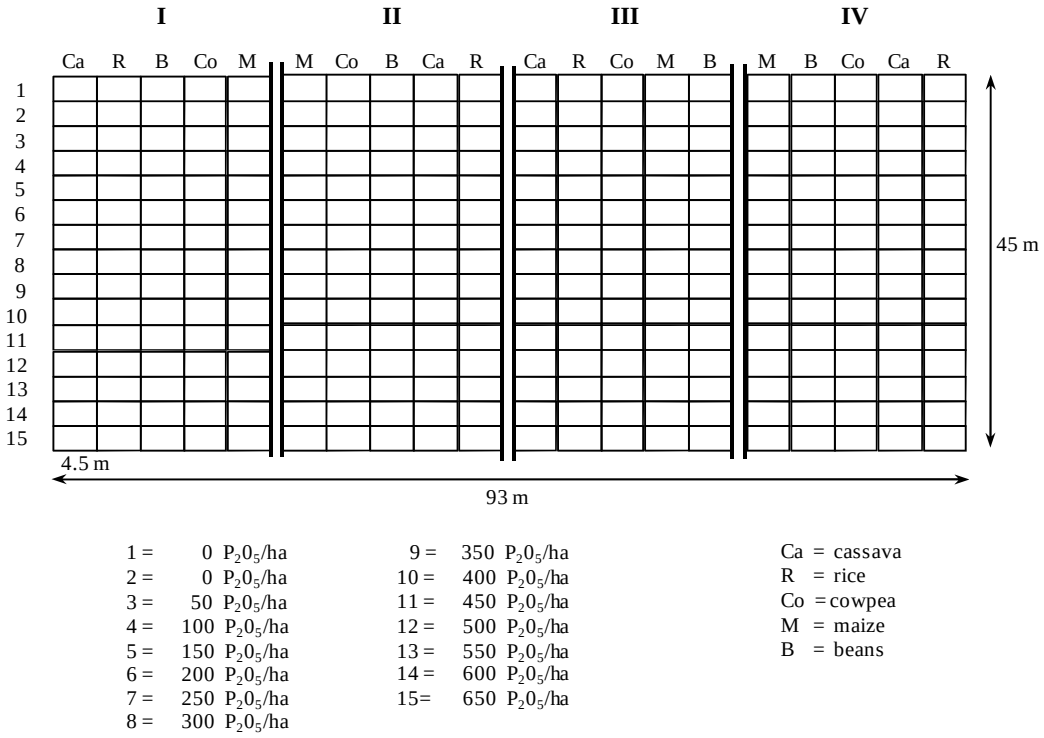


Figure 8. Systematic design to study the response of several crops to the application of increasing levels of P.

Lime, gypsum, rock phosphates, basic slag and manures need good contact with the soil to dissolve or decompose in order that the nutrients become available to the plants. For that reason they are normally applied broadcast uniformly over the entire plot or experiment and then incorporated into the soil during land preparation and before planting. The disadvantage of this method of application is that weeds also benefit from the fertilizers or amendments applied.

In case of cassava, most water-soluble chemical fertilizers should be applied either at time of planting or at about one month after planting (MAP). In case of horizontally planted stakes, the fertilizers are generally applied after the young plants have emerged from the soil. Plants need phosphorus (P) mainly at the early stages of growth, so most P-sources are applied at or shortly (1 month) after planting. Nitrogen (N) and potassium (K) can also be all applied at or shortly after planting, or the applications can be split with half applied at or shortly after planting and the other half at 2-3 MAP. Applying fertilizers at a later stage is generally less effective.

Micronutrients such as zinc (Zn) and iron (Fe) can be applied (if necessary) to the soil as sulfates or chelates at time of planting, but in high-pH or calcareous soils these fertilizers should be applied to the leaves as a spray at 2, 3, and 4 MAP. These nutrients can also be applied by soaking the stakes for 15 minutes in a solution of 2-4% $ZnSO_4.7H_2O$ or $FeSO_4.7H_2O$ before planting.

When fertilizers, lime or manure need to be applied in an experiment, these are usually weighed out in separate plastic bags for each plot before going to the field. In the field, these bags are laid out in each plot, either uniformly to all plots or according to specific treatments. Before application it is important to check that every plot has the correct number and types of fertilizers. After this check, the lime or manure could be mixed and applied broadcast over the entire plot and then incorporated into the soil with a hoe or hand tractor. The bags of chemical fertilizers are emptied into a pail and thoroughly mixed, after which a small amount of the mixture is applied in short bands or holes previously made along-side each planted stake or young plant, making sure that each plant receives more or less the same amount of fertilizer. If, after all plants in the plot have received fertilizer there is still some left in the pail, this remaining fertilizer should be distributed again evenly over all plants in the plot until all the fertilizer has been applied. Once this is finished, the fertilizers in the holes or bands should be covered with soil. Applying fertilizers evenly over all the plants in the plot requires considerable experience by the field workers.

Weeding

Cassava is a poor competitor and suffers greatly from competition from weeds or other crops growing nearby, especially during the early stages of growth. This early growth is also quite slow as compared to many other crops like maize, rice and beans. For that reason, weeds should be eliminated during the first 3-4 MAP and intercrops should be planted at least 30 cm away from the young cassava plants. Once the cassava canopy closes, most weeds will be shaded out, and in general no more weeding is necessary after 3-4 months. If leaf drop during the later growth stages is severe and weeds reappear, these might be cut off by machete to prevent weeds from flowering and reseeding. Weeding at this late stage is unlikely to increase root yields and may damage the swollen roots if weeding is done by hoe. Thus, hand weeding by hoe should start at about 3-4 WAP and is followed by another 1-2 weedings at 2 and 3 MAP.

Band application of fertilizers can markedly speed up the cassava canopy formation and thus reduce the need for additional weeding. On the other hand, application of cow or goat manure can increase the weed problem as many weed seeds will pass through the animal's gut and will germinate when the manure is broadcast and incorporated.

When labor is scarce or expensive, weeds can also be controlled by spraying of pre-emergence herbicides such as diuron, alachlor, oxifluorfen and metholachlor right after planting (even over the vertically planted stakes); this can be followed by hand weeding or by application of post-emergence herbicides like paraquat and glyphosphate when weeds reappear at 2 to 3 MAP; the latter herbicides should be applied using a plastic or metal shield over the nozzle to prevent hitting the cassava leaves or stems.

Determination of Yield in Cassava Monoculture and When Intercropped

Determining the effective plot when cassava is planted in monoculture and at the same plant spacing is quite simple, as shown in **Figure 9**. Usually one border row along all four sides of the plot is excluded, and only plants within the remaining center part of the plot, i.e. the "effective plot" are harvested and the root (and top?) weight determined. The

root yield of the plot in t/ha is calculated as the root weight (in kg) in the effective plot x 10 divided by the area of the "effective plot" (in m^2).

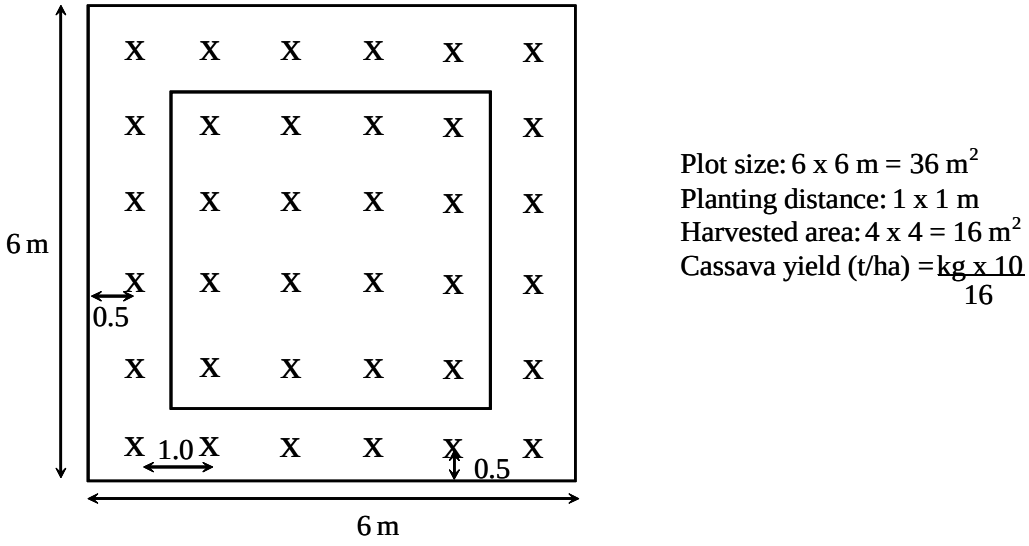
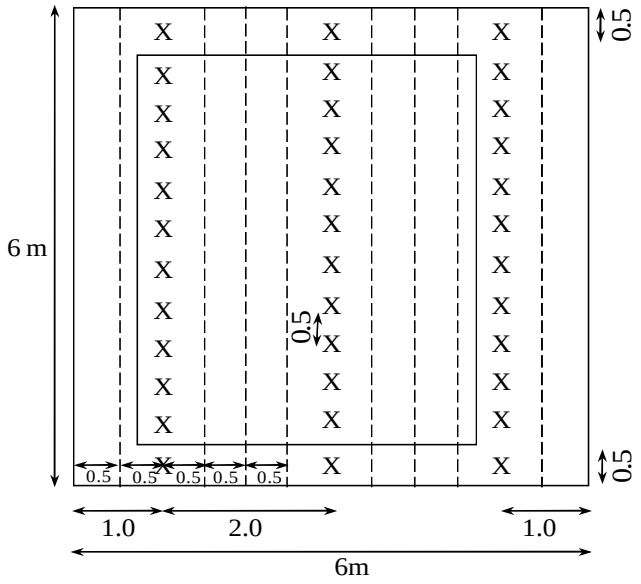


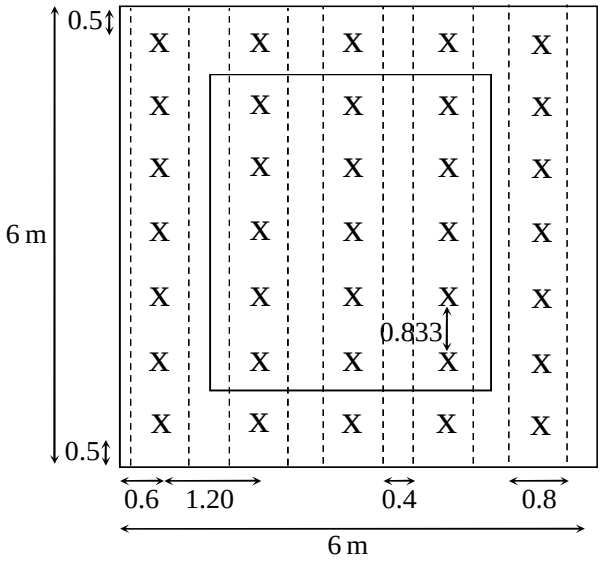
Figure 9. Plot lay-out when cassava is grown in monoculture.

When cassava is intercropped the space between rows is often widened, while the space between plants in the rows is shortened to maintain a cassava population of 10,000 plants per ha, while accommodating one, two or three rows of intercrops between the cassava rows (**Figures 10** and **11**). To determine the yields of both cassava and the intercrops, it is important to determine the correct area of the effective plot to be harvested. The effective plot should always exclude at least one border row, and include the same ratio of cassava to intercrop rows as you would find in the larger field. Thus, **Figure 10** shows that if one row of cassava is alternated with three rows of upland rice, the effective plot may include two rows of cassava and six rows of upland rice, and the harvested area for both crops would be 4 x 5 = 20 m^2. In **Figure 11**, if cassava is intercropped with two rows of peanut, the effective plot could include three rows of cassava and six rows of peanut, and the harvested area is 3.6 x 4.165 = 15 m^2. **Figure 12** is an example of an alley cropping trial in which one out of every five rows of cassava is replaced by one hedgerow of *Leucaena leucocephala*. To maintain a constant cassava population of 10,000 plants per ha, the plant spacing within the row is reduced to 0.8 m. In this case the effective plot should include one hedgerow of *Leucaena* and four rows of cassava, and the harvested area is 4.8 x 5.0 = 24 m^2. In order to accommodate two hedgerows and six cassava rows the plot size had to be increased to 6 x 8 meters.



Plot size: 6 x 6 m = 36 m^2
Cassava planting distance: 2.0 x 0.5 m
Rice planting distance: 0.5 m between rows
Harvested area: 4 x 5 = 20 m^2
Cassava yield (t/ha) = $\frac{\text{kg x 10}}{20}$
Rice yield (t/ha) = $\frac{\text{kg x 10}}{20}$

Figure 10. Plot lay-out when cassava is intercropped with three rows of upland rice.



Plot size: 6 x 6 m = 36 m^2
Cassava planting distance: 1.20 x 0.833 m
Peanut planting distance: 0.4 m between rows
Harvested area: 3.6 x 4.165 = 15 m^2
Cassava yield (t/ha) = $\frac{\text{kg x 10}}{15}$
Peanut yield (t/ha) = $\frac{\text{kg x 10}}{15}$

Figure 11. Plot lay-out when cassava is intercropped with two rows of peanut..

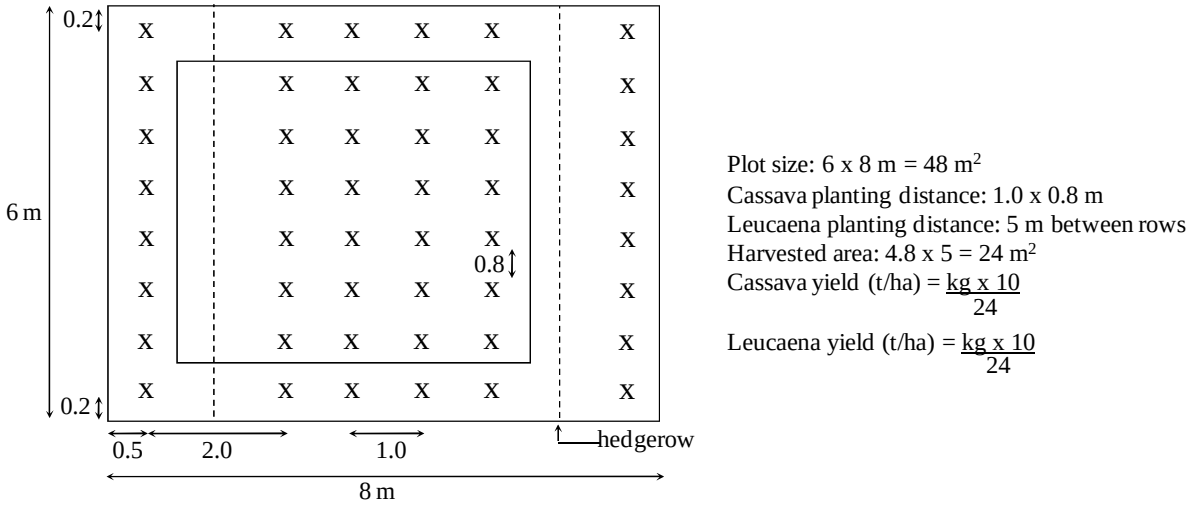


Figure 12. Plot lay-out when four rows of cassava are grown in alleys between hedgerows of Leucaena leucocephala.

Determination of Yield When Some Plants are Missing

Since cassava experiments generally have relatively few plants in each plot, it is very important to try to have a complete plant stand, especially inside the effective plot that is used to measure the yield. However, sometimes one or more plants may be missing because they did not germinate, were attacked by termites or rogued out because of CMD. If we try to correct for missing plants by multiplying the weight of the roots of the remaining plants in the effective plot by the number of plants that should have been harvested divided by the number of plants actually harvested, we tend to grossly overestimate the actual yield. This is because cassava plants surrounding the missing hill have less competition and will thus have higher yields than those that are completely surrounded by eight other plants. Similarly, statistical methods of estimating the yield of missing plants tend to overestimate those yields as cassava plants adjacent to missing hills generally have higher yields because they have more space to grow. Research conducted in the Philippines indicate that plants adjacent to missing hills (treatments 2 and 6 in **Table 4**) had substantially higher yields than those in plots without missing hills (treatment 1 in **Table 4**), and that cassava yields in plots with up to 30% missing hills were not significantly different from those of plots without missing hills, because the higher yields of plants adjacent to the missing hills compensated for the missing plants (**Table 6**). These results were independent of the variety, the plant population or fertilizers used. Thus, when up to 30% of plants in the effective plot are missing, the root weight (in kg) of the remaining plants x 10 divided by the area (in m^2) of the effective plot will give the best estimate of actual yields (in t/ha). If more than 30% of plants are missing the yield data obtained should probably not be used; alternatively, some border row plants could be harvested and weighed to be included with the harvested plants in order to complete up to 70% of a complete plant stand in the effective plot. If more than 50% of plants in the effective plot are missing, then the yield data of those plots should not be used. In any case, it is always useful to count and record the actual number of harvested plants in the effective plot as this may explain some of the observed yield differences.

In some cases plants are missing because they were stolen shortly before the experiment was harvested, or plants were uprooted and the roots damaged by wild pigs. In that case the plants surrounding the missing hills would not have benefitted from reduced competition during the growth cycle and thus would not have increased yields. To determine the yield of the plot, the root weight of the remaining plants in the plot could therefore be corrected for those missing hills that were stolen or damaged shortly before the harvest.

Table 6. Summary of results of experiments on the effect of missing hills on yield as influenced by variety, population and fertilization, in ViSCA, Leyte, Philippines.

Treatments	Yield (t/ha)	Treatments	Yield (t/ha)	Treatments	Yield (t/ha)
Variety (NS)		Population (NS)		Fertilizer (NS)	
Golden Yellow	22.75 a	10,000 pl/ha	11.04 a	00-00-00	26.26 a
CMC-40	22.61 a	20,000 pl/ha	11.50 a	25-25-25	30.57 a
		40,000 pl/ha	9.12 b	50-50-50	26.31 a
CV (%)	14.59	CV (%)	9.52	CV (%)	17.27
Missing hills (NS)		Missing hills (NS)		Missing hills (*)	
0%	24.68 a	0%	10.98 a	0%	30.23 a
10%	24.17 a	10%	10.36 a	30%	28.54 a
20%	26.48 a	20%	10.11 a	35%	25.88 b
30%	21.38 a	30%	10.77 a	40%	26.21 b
CV (%)	15.95	CV (%)	17.28	CV (%)	11.53
Interaction (NS)		Interaction (NS)		Interaction (NS)	

Mean separation (LSD, 0.05)
Source: *Villamayor, 1988*

On-station, On-farm and Farmer Participatory Research (FPR) Trials

In **on-station experiments** researchers design and manage the trial with their own trained personnel and thus maintain full control over every aspect of the experiment. The experiment should have enough replications to obtain reliable results and to be able to analyze the data statistically. But if soil or climatic conditions in the cassava growing areas differ from those in the experiment station (in many stations soil fertility is much higher than in farmers' fields due to repeated use of fertilizers or manures), then it may be better to conduct **on-farm experiments** in farmers' fields that are more representative of the agro-ecological conditions in which much of the cassava is grown. These experiments are still designed and mostly managed by researchers although the farmer may be paid for the land and for maintaining the trial free of weeds. The results are used by the researchers and the planting material produced is often taken away while the farmer receives the roots for their own consumption of for sale. These experiments also have replications and the data can usually be analyzed statistically.

In contrast, **farmer participatory research (FPR) trials** are designed and managed by volunteer farmers, who are also the owners of the trials and the owners of the results, the roots and the planting material produced. Usually, researchers or extension workers first discuss with the farmers of a village (or pilot site) about cassava, how it is grown, what it is used for, and what might be the main problems for increasing yields. After this, farmers may want to visit some experiments at an experiment station or in another famer's field to see and discuss possible solutions to their problems. They could be encouraged to test some of those solutions as treatments in simple FPR trials on their own fields in order to select the best varieties or practices. Researchers or extension workers should discuss and help farmers design these trials. Most of these trials have only 5-8 treatments with one being the farmer's traditional variety or practice. In each trial only one factor should vary among treatments while all other factors remain constant. Thus, in an FPR variety trial the treatments consist of different varieties, while fertilization, weeding etc remains the same for all treatments. Similarly, in an FPR fertilizer trial the treatments consist of different levels of NPK fertilizers or may have different combinations of N, P or K fertilizers, but the variety and other practices are the same throughout the trial. These trials generally have small plots (see **Figure 9** for monoculture, or **Figures 10** or **11** for intercropping) and no replications. If several farmers in the village conduct the same type of trial and all agree to use the same treatments, then each of these trials can be considered a replication. By calculating the average yields of each treatment across these trials, the results obtained become more reliable and more convincing.

At time of harvest, researchers or extension workers harvest the trials together with the participating farmers. The roots of plants harvested in the effective plots are weighed and the weights recorded, while border rows remain standing. The harvested roots are left in a pile in the center of the plot with a sign indicating the root yield (and sometimes starch content).

The following day, a farmers' field day may be organized with participation of other farmers from the village or from surrounding villages. Farmers are briefed about the objective of the field day and the type of trials that have been conducted. They then receive sheets with the lay-out of the various types of trials; they are asked to write down on those sheets their evaluation of each treatment (1 = very bad; 2 = OK; and 3 = very good) when they visit the trials. These evaluations may be based on the root yields shown with each pile of harvested roots; on the root size, shape and color; on the taste of raw roots; on the plant type of the plants still standing in the border rows, or on any other criteria farmers use. After visiting all the trials conducted in the village, the treatments and their average yields are shown on a large sheet of paper and the results discussed. For every treatment farmers are asked to raise their hands if they had scored the treatment as "very good" (3). The number of raised hands are quickly counted and written down on the chart with results. The treatments with the highest scores are obviously the most preferred. Reasons for farmers' preferences should be discussed. Besides data on yield (and starch content?) it is often useful to show the gross income from yields obtained, the estimated costs and the resulting net income of each treatment. Farmers who sell their harvested roots generally prefer those treatments with the highest net income.

Thus, in FPR trials, farmers design and conduct the trials, they make the final selection of the most suitable varieties and production practices. Researchers and extension workers help farmers in identifying and prioritizing their own problems, suggesting

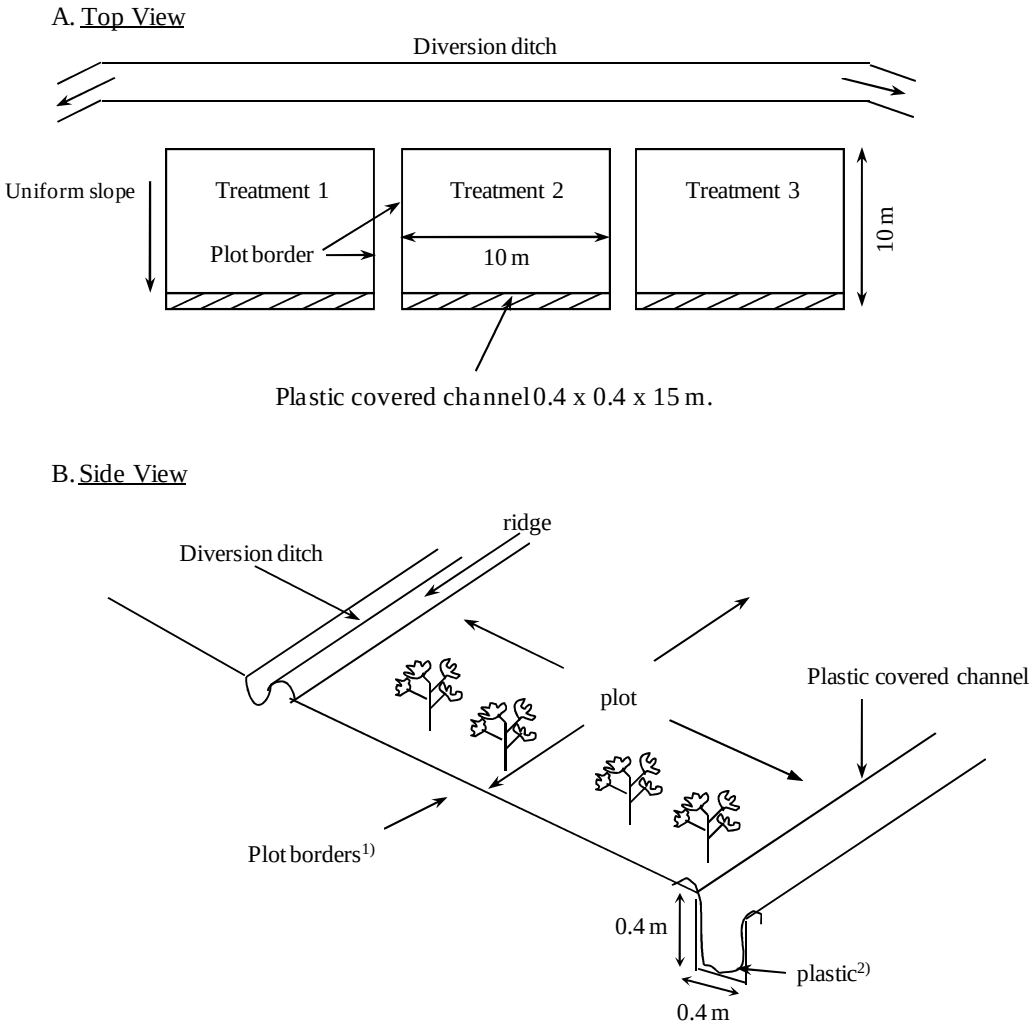
possible solutions, discussing designs, setting out the trials, solve any unexpected problems, and finally harvest the trials and discuss the results. Researchers and extensionists do not promote or recommend any particular treatments, but let farmers make their own decisions and their own selections. Farmers are more likely to adopt those varieties or practices that they themselves have tested and selected as the most suitable for their own conditions.

Simple Erosion Control Trials on Farmers' Fields

To determine the effect of particular treatments on soil losses by erosion, researchers have generally done erosion control trials using "runoff plots" on a uniform slope in an experiment station. These experiments are expensive in terms of equipment used, and are labor intensive because soil losses and water runoff need to be determined after each rainfall event. To determine how certain agronomic practices effect soil losses by erosion, a much simpler method can be used that determines only the amount of eroded soil in each treatment by weighing the amount of wet eroded soil that is trapped in a plastic covered trench dug along the entire bottom edge of each plot. The amount of runoff water is not determined as the runoff is allowed to seep away through small holes made in the plastic. The eroded wet soil collects on top of the plastic and can be dug out and weighed every month or 2-3 times during the crop's growth cycle, mainly during the rainy season. After weighing the wet eroded soil, a sample of 1-2 kg is taken to be dried to determine the percent dry soil in the original sample. The amount of dry soil loss (t/ha) can be estimated from the weight of wet soil collected and the dry matter (DM) content of the wet sample as follows: dry soil loss (t/ha) = wet soil (in kg/plot) x % DM/100 times 10 divided by the plot size (in m^2). One can plot the accumulated dry soil loss against time, from planting to the final harvest, in order to see when most soil loss by erosion occurred.

To get rather accurate data one must take certain precautions:

1. The trial must be laid out on as uniform a slope as possible and plots are laid out side by side perpendicular to the slope, i.e. on the contour. If there are many plots these can also be laid out in 2-3 rows perpendicular to the slope as long as each plot has more or less the same slope. An example of such a trial is shown in **Figure 13.**
2. It is very important that the soil-collecting ditches are laid out exactly on the contour so that all runoff water will flow naturally into the ditches and not enter or leave the plot through side borders. If the contour line is not straight but curved, the ditches also need to curve to follow the contour. If the slope is not uniform and uni-directional, the plots may end up with curved borders and be trapezoidal rather than square or rectangular. This does not matter as long as we can more or less accurately determine the size of each plot.
3. The erosion must be caused only by the rainfall falling on the plot and not by runoff coming into the plot from the area upslope from the plot. To achieve this, a diversion ditch is dug along the upper side of the experiment, so that runoff water from above slope is diverted away and does not enter the experimental plots. To prevent runoff water entering or leaving through the side borders (which happens when the plots are not exactly on the contour), this can be prevented by building a soil ridge or by digging in a metal sheet along each side border.



1) Plot borders of sheet metal, wood or soil ridge to prevent water, entering or leaving plots.
2) polyethylene or PVC plastic sheet with small holes in bottom to catch eroded soil sediments but allow run-off water to seep away. Sediments are collected and weighed once a month.

Figure 13. Experimental lay-out of simple trials to determined the effect of soil/crop management practices on soil erosion.

4. The plastic-covered channel should be able to accommodate all the eroded soil and runoff water resulting from a heavy rainstorm. Usually a 40x40 cm channel along the entire lower side of the plot is sufficient. The ditch is covered with plastic sheet of 1.5-2.0 m width. The side edges are dug into the soil as shown in **Figure 13**. PVC plastic (used for shower curtains) generally last longer than poly-ethylene plastic. Exposed to the sun the plastic may deteriorate after a while. A few holes or tears are not a problem as runoff water is allowed to seep out any way, but if the plastic deteriorates too much it

may need to be replaced. If runoff water does not seep out within a few days after a heavy rainstorm, it may be necessary to make additional holes in the bottom of the plastic sheet with a nail or pointed stick. If the channel is 40-50% full with eroded soil, this soil should be dug out and weighed so as to accommodate additional soil and run-off water during the next rain storm without danger the channel will overflow. If the channel is not on the contour, soil and water will accumulate at one end with the possibility that soil and water will overflow at that end.

5. At time of harvest, cassava plants in the effective plot (excluding one border row) are harvested and the yield is determined according to the size of the effective plot. In case contour hedgerows are planted as an erosion control treatment, these hedgerows as well as the adjacent cassava row(s) should be included as part of the effective plot, since they occupy space in the farmer's field; moreover, the hedgerows sometimes compete with the adjacent cassava rows and reduce their yield.
6. In case of FPR erosion control trials, the number of treatments (plots) should be limited to five or six, one of which is the farmer's traditional practice. The advantage of conducting these trials on farmers' fields is that farmers can see clearly which practices (treatments) are most effective in reducing soil losses by erosion by looking at the amount of eroded soil in each plastic-covered channel. Once they see how much soil (including water and fertilizers) they are losing each year, they will want to adopt those practices that are effective in reducing erosion while requiring little additional money or labor. For that reason, the gross income, total production costs and net income, as well as the soil loss, should be calculated and shown to farmers for each treatment, so farmers can make an informed choice about which erosion control practices to adopt.

Measuring the % Slope of a Piece of Land

Figure 14 shows an easy way of measuring slope using a "line level"; this is basically a small carpenter level that has two hooks for hanging on a horizontal string. The string is exactly horizontal when the air bubble is between the lines indicated on the leveling device. One person holds one end of a 2-meter string on the soil surface, while a second person holds the other end of the string against a vertical pole (can be the handle of a hoe) and moves that end up or down until the carpenter's level indicates that the string is exactly horizontal. The distance (in cm) from the string on the pole to the foot of the pole (a in **Figure 14**) divided by b (= 200 cm) times 100 is equal to the % slope of the land.

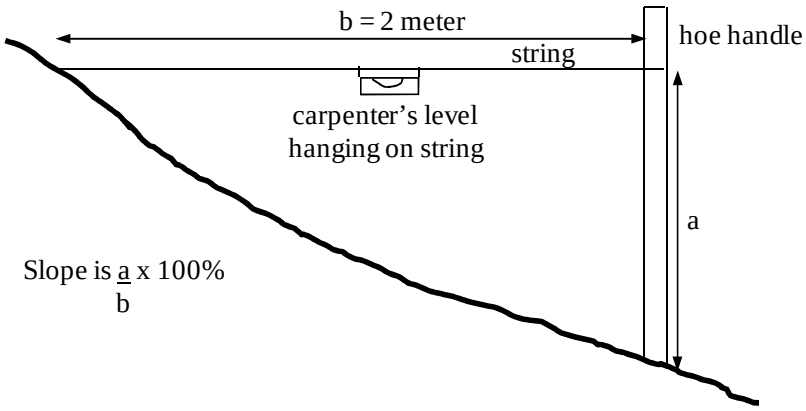


Figure 14. Simple method to determine the slope using a line level.

Setting out Contour Lines

Most people are familiar with the A-frame (**Figure 15**) to set out contour lines. One leg of the A-frame is placed next to a stake placed to mark the beginning of the contour line, while the other leg is moved side ways until the string touches the mark on the horizontal bar of the A-frame. At that point the two legs are level; a second stake is placed to mark the position of the second leg. This leg stays next to the second stake while the first leg is swayed around to find the third point of the contour line. The process is repeated over and over again until the whole contour line has been marked. The advantage of the A-frame is that it can be built from commonly used materials, such as wooden posts, string and a stone used as weight. However this method is time consuming and will not work well if the soil surface is rough or the path of the contour line is obstructed by weeds, bushes or trees.

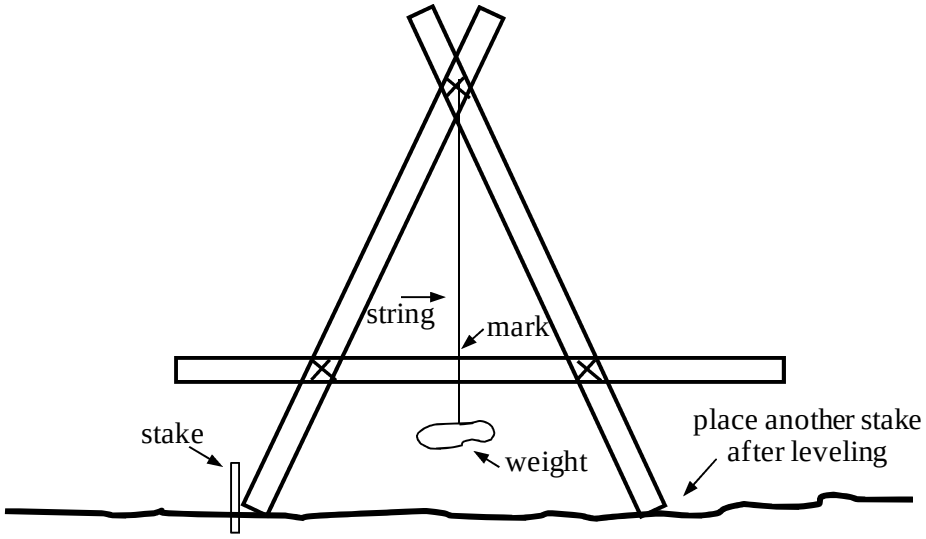


Figure 15. A-frame to set out contour lines.

Figure 16 shows an easier way using again a line level hung on a 10-20 m long string. Two poles (or hoe handles) are both marked at the same height, say 1 meter. One pole is placed next to a stake placed to indicate the beginning of the contour line and a person holds one end of the string on the 1-meter mark. A second person moves 10-20 m away holding the other end of the string on the 1-meter mark on his/her pole. With the string tightened a third person watches the line level hanging on the string between the two poles, signaling to the second person to move the second pole up or down slope until the line level indicates that the string is exactly horizontal. A second stake is placed at the foot of the second pole to indicate that the first two stakes are on the same level. The first person now moves his/her pole to the second stake and the process is repeated until the whole contour line has been marked. This method is much faster as the length of the string can be varied depending on the roughness of the terrain, and the string can go around obstacles in the path of the contour line.

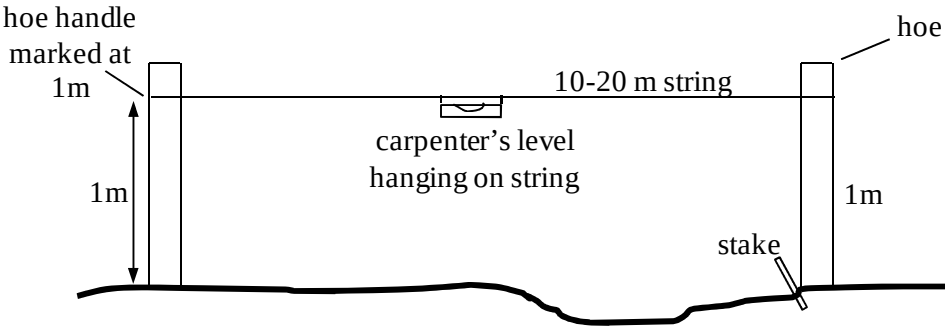


Figure 16. Simple method to set out contour lines using a line level.

Figure 17 shows a third method (called "buffalo horn") using a clear plastic tube which is half filled with water. The tube is tied to a vertical pole marked at eye level, say at 1.6 m, so that the water level is at the height of the mark. A second pole is marked at the same level as the first. A second person moves the second pole about 10-20 m away. The first person signals to the second person to move the pole up or down slope until he/she can see the mark on the second pole exactly across the two water levels in the plastic tube. In that case the position of the two poles are on the same level in the landscape. These can be marked by placing stakes; the process is repeated until the whole contour line has been marked.

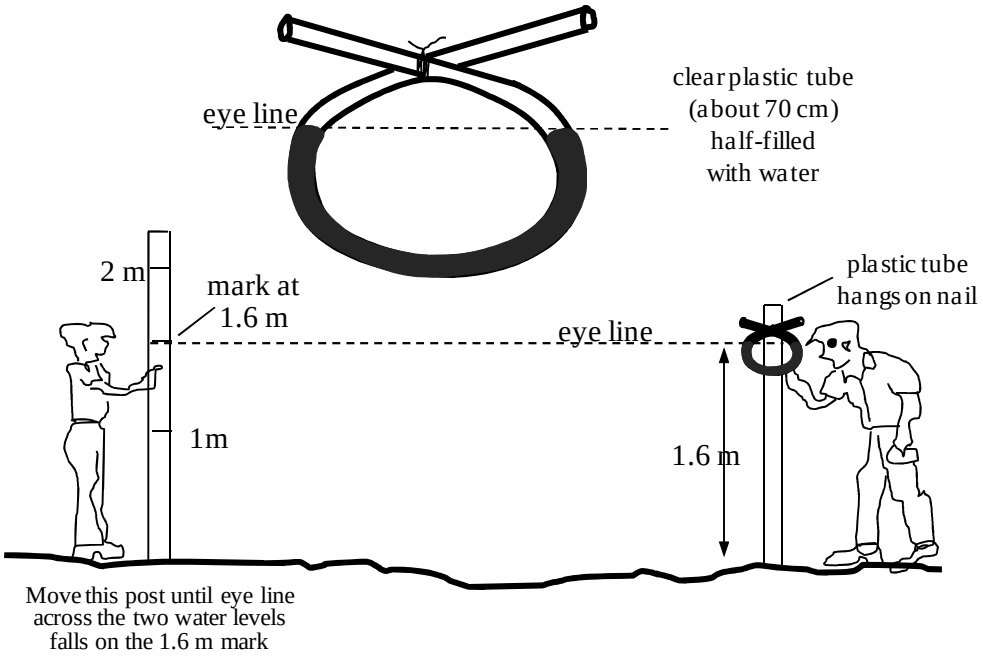


Figure 17. Simple method to set out contour lines using a plastic tube with water: "buffalo horn".

Determination of the Starch Content of Cassava Roots

The per cent starch or dry matter (DM) in cassava roots can be determined or calculated rather quickly from the specific gravity of the roots. The higher the specific gravity (kg/liter) the higher the starch and DM contents of the fresh roots. The specific gravity can be determined by weighing a certain amount of fresh roots in air and then weighing the same roots completely submerged under water. Many starch factories use a special starch balance, with which they first weigh exactly 5 kg of fresh roots in a basket hanging in air, and then the same roots in a second basket hanging in water. A second scale of the same balance indicates both the weight of the root sample under water and the starch content. Farmers get paid a certain price according to the starch content of the roots.

While a specially made starch balance is convenient, it is not essential. The same methodology can be applied using two different balances, one of 5 or 10 kg capacity to weigh in the air about 5 kg of fresh roots (cut in smaller chunks) placed in a nylon screen bag. After recording the exact weight of the roots (anywhere between 4 and 6 kg), the bag with roots is hung on a hanging scale of 1000 gm capacity while being completely submerged under water, without touching the bottom or sides of the container, such as a plastic garbage can filled with water. The second balance indicates the weight of the cassava roots under water; this tends to be about 10-15% of its weight in air. Thus, a 5 kg sample of fresh cassava roots may weigh anywhere between 500 and 650 gm when completely submerged under water. The starch or DM contents can be calculated as follows:

$$\text{Specific gravity } X = \frac{\text{Weight in air}}{\text{Weight in air - Weight in water}}$$

the starch content = 210.8 X – 213.4
the DM content = 158.3 X – 142.0
and the starch content = 1.33165 x (% DM) – 24.306

As an example: fresh roots of a certain variety are cut into smaller chunks, put in a nylon screen bag and the bag is weighed on a normal kitchen balance. The weight is 4.53 kg or 4530 g. When the same bag of roots is completely submerged under water and weighed again with the hanging scale, its weight is now only 550 gm. In that case the specific gravity of the roots is

$$X = \frac{4530}{4530\text{-}550} = \frac{4530}{3980} = 1.1382 \text{ kg/liter}$$

the starch content of the roots = (210.8 x 1.1382) – 213.4 = 26.53%
and the DM content = (158.3 x 1.1382) – 142.0 = 38.18%

With this simple method we can rapidly determine the starch and DM contents of the roots; the higher the starch or DM contents are the more valuable the roots are for the starch, animal feed or ethanol industries, and thus the higher the price that they are willing to pay for the roots. For that reason, cassava breeders will normally select those varieties

having both high yield and high starch or DM contents. To obtain accurate data it is important to tare the balances with the empty baskets or nylon bag in the air or in water.

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CHAPTER 14

DRY MATTER ACCUMULATION AND NUTRIENT ABSORPTION AND DISTRIBUTION DURING THE GROWTH CYCLE OF CASSAVA

Reinhardt Howeler[1]

INTRODUCTION

Cassava (*Manihot esculanta* Crantz) is grown mainly by small-holder farmers in tropical countries, but can also be grown in subtropical climates up to a latitude of about 30° N and S of the equator. In tropical countries the crop can be grown from sea level up to about 2000 m above sea level. It can also grow in areas with heavy rainfall as well as in areas with long dry seasons with only about 800 mm annual rainfall. The crop is extremely drought tolerant and plants will never die because of drought, except possibly during the first 1-2 months after planting, when adequate soil moisture is necessary for good establishment. Once established the plant can tolerate drought by slowing its growth and dropping some of the older leaves. Once it starts raining again, new leaves and roots are formed rapidly and growth resumes and dry matter (DM) continues to accumulate in the tuberous roots and other plant parts. However, cassava does not tolerate flooding for more than a few days and will not grow well in poorly drained or waterlogged soils. Under these conditions, leaves will droop or fall off and the plants may eventually die.

Cassava is also tolerant of very acid and low-fertility soils and will still produce reasonably well in these soils where most other food crops would fail. Since the crop has this ability to grow on poor soils (Cock and Howeler, 1978), many people think that cassava does not need and will not respond to fertilization. This, however, is not the case. Cassava responds to balanced fertilization as well as, if not more so, than many other crops (FAO, 1980), and this fertilization tends to be highly economic, as indicated by the high Value-Cost Ratio (VCR) of fertilizing cassava as compared to fertilizing other major crops (Richards, 1979).

Thus, cassava can grow under a wide range of climate and soil conditions, but how well it will grow will depend on the particular conditions under which it is grown, and on the crop/soil management it is receiving during the cropping cycle. These conditions also affect the rate of nutrient absorption, the distribution of each nutrient among various plant parts and how much of each nutrient is actually removed from the field at harvest. The latter will determine the rate of nutrient depletion of the soil and the need to replenish those nutrients in order to maintain high yields and the sustainability of the cropping system.

Cassava Growth and Nutrient Uptake under Subtropical Conditions in Argentina

Orioli *et al* (1967) determined the accumulation and distribution among various plant parts of dry matter (DM) and N, P, K, Ca and Mg during the first six months of the growth cycle of cassava in northeast Argentina. During the sixth month cassava growth slowed down, probably due to the onset of winter. DM and nutrients in roots, leaves and stems were determined by bi-weekly sampling and analysis of these various plant parts. **Figure 1** shows the accumulation and distribution of DM and N in the roots, leaves and stems during the 6-month growth cycle, both under fertilized and unfertilized conditions.

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DM production was slow during the first two months, but increased rapidly during the next 2-3 months, before slowing down again during the sixth month. Roots accumulated DM at a rather constant rate from the 3rd to the 6th month, while leaves and stems accumulated little during the sixth month due to leaf fall and the onset of colder weather. Fertilized plants accumulated DM in much greater quantities than unfertilized plants, but the relative distribution among plant parts was similar under both conditions. The rate of N accumulation was also very low during the first two months, reached a maximum during the third and fourth months and then slowed down during the last two months (**Figure 1B**). The non-fertilized plants even lost N during the final two months. Although the DM was fairly evenly distributed among the three plant parts at six months of growth, at this stage of rapid vegetative growth N had accumulated mostly in the leaves, followed by stems and roots. This reflects the high protein content of leaves as compared to roots and stems. The rate of accumulation of P and K followed a similar pattern as that of N. Again, at six months most of the absorbed P and K was present in the leaves. Calcium accumulation in leaves and roots stopped after three months, while that in stems continued. The relative nutrient accumulation curves for fertilized and unfertilized plants were similar, although the fertilized plants absorbed nutrients in much greater quantities.

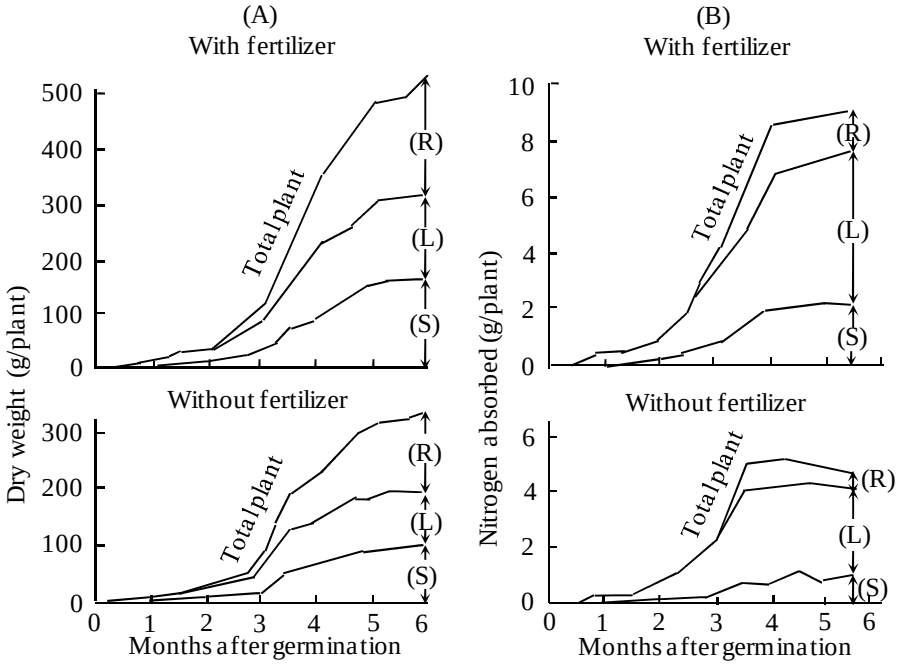

Figure 1. The accumulation and distribution of dry matter (A) and nitrogen (B) in the roots (R), leaves (L) and stems (S) of cassava during the first six months of growth, with and without fertilization (monthly application of 20 kg N, 8 kg P and 16 kg K/ha) in Argentina. ***Source:*** *Orioli et al., 1967.*

Cassava Growth and Nutrient Uptake under Tropical Conditions in Indonesia

Nijholt (1935) reported the dry matter and nutrient accumulation and distribution of two cassava varieties grown for 14 months in Bogor, Indonesia. **Figure 2** shows that the rate of DM accumulation was fairly constant between the 2nd and 12th month, but slowed down during the last two months of the growth cycle. The tuberous roots became the dominant DM sink after the 3rd month, followed by the stems and leaves. At time of harvest at 14 months after planting (MAP) 66% of total DM was in the roots, 31% in stems and only 2% in the leaves. **Table 1** shows the concentrations of N, P, K, Ca and Mg in the leaves, stems and roots at 2-month intervals of the growth cycle. Concentrations of N, P and K decreased with increasing age of the plant in all three plant parts, while the Ca concentration increased in the leaves but decreased in stems and roots.

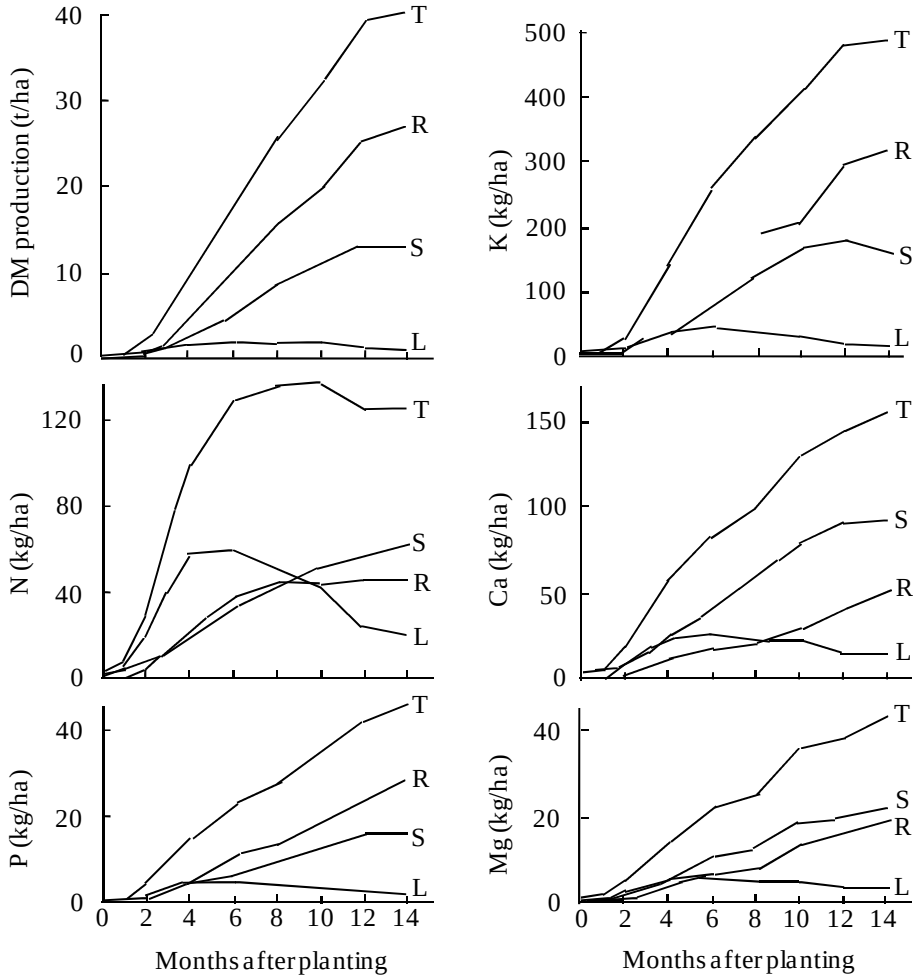

Figure 2. The accumulation of dry matter, N, P, K, Ca and Mg in leaves (L), stems (S), roots (R) and the total plant (T) of cassava, cv. São Pedro Preto, during a 14-month growth cycle in Indonesia.

***Source**: Adapted from Nijholt, 1935.*

Table 1. Nutrient concentration of leaves, stems and roots of cassava, cv. São Pedro Preto, at various ages in Indonesia.

	Leaves (% of DM)					Stems (% of DM)					Roots (% of DM)				
Month	N	P	K	Ca	Mg	N	P	K	Ca	Mg	N	P	K	Ca	Mg
2	3.28	0.29	2.21	1.13	0.33	0.88	0.27	1.96	1.07	0.30	1.03	0.19	2.13	0.48	0.16
4	3.41	0.27	2.05	1.38	0.28	0.81	0.21	1.69	1.03	0.27	0.45	0.11	1.47	0.22	0.07
6	3.06	0.24	2.11	1.37	0.27	0.64	0.13	1.53	0.78	0.20	0.36	0.11	1.41	0.16	0.06
8	3.20	0.24	2.16	1.43	0.28	0.49	0.12	1.52	0.69	0.15	0.28	0.09	1.18	0.13	0.05
10	2.79	0.22	2.00	1.39	0.28	0.48	0.12	1.53	0.73	0.17	0.22	0.10	1.07	0.15	0.07
12	2.47	0.23	1.61	1.48	0.29	0.44	0.12	1.38	0.70	0.15	0.18	0.09	1.14	0.16	0.06
14	2.34	0.23	1.33	1.61	0.35	0.48	0.12	1.26	0.72	0.17	0.17	0.11	1.19	0.19	0.07

Source: *Adapted from Nijholt, 1935.*

Figure 2 also shows the accumulation of these five nutrients during the growth cycle and their distribution among leaves, stems and roots. Nitrogen initially accumulated mainly in the leaves until the sixth month, after which it declined in the leaves due to leaf fall, but continued to increase in stems and remained constant in roots. At time of harvest about 49% of total absorbed N was found in stems, 36% in roots, and only 15% was found in leaves. A similar distribution was observed for Ca and Mg. In contrast, P and especially K accumulated mainly in the roots, followed by stems and leaves. At harvest about 62% P and 65% of K was found in the roots, 34% of P and 33% of K in stems, and only 4% of P and 2% of K in the leaves. This distribution can vary considerably depending on variety, soil and climatic conditions as well as the age of the plant at harvest. In this case, the K content was highest in the total plant as well as in the roots, followed by Ca, N, P and Mg. The fresh root yield at 14 months was 64.7 t/ha.

Cassava Growth and Nutrient Uptake under Tropical Condition in Two Locations of Colombia

In order to further elucidate the accumulation and distribution of DM and nutrients during the cassava growth cycle, and the effect of climatic conditions and soil fertility, four experiments were conducted between 1978 and 1984 at two experiment stations in Colombia. The first two trials were conducted in 1978/79 and 1982/83 at the CIAT-Quilichao station in Cauca Department, located at nearly 1000 masl and about 4° north of the equator. Another two trials were conducted in 1983/84 and 1984/85 at the ICA-Carimagna experiment station in the Eastern Plains (Llanos Orientales) of Colombia, located at about 300 masl in a vast area of very acid and infertile soils. The Quilichao site has a bimodal rainfall distribution, while the rainfall in Carimagua is mono-modal with an intense dry season from Nov/Dec to March/April. The soil conditions of both sites are shown in **Table 2**. The soil in Quilichao is quite acid, with a high level of organic matter (OM), low in available P, but with reasonable levels of Ca, Mg and K. The soil in Carimagua is extremely acid, with medium levels of OM, very low in P, Ca, Mg and K and having a high Al saturation. For normal growth at this site most crops require the application of 6 t/ha of lime, but cassava grows well with 0.5-2.0 t/ha of lime.

Table 2. Soil fertility characterization of soils where cassava experiments were conducted in CIAT-Ouilichao in 1978/79 and 1982/83, and in Carimagne in 1983/84 and 1984/85.

			(%)	(ppm)	-------(me/100 gm)------				(%)	-------(ppm)-----		
Site	Date	pH	OM	P	Ca	Mg	K	Al	Al sat.	B	Zn	Mn
CIAT-Quilichao (before liming)	05/05/78	4.6	8.5	2.3	0.92	0.58	0.41	3.1	62	0.14	1.4	26
CIAT-Quilichao (after liming)	06/09/78	4.4	-	5.5	1.29	1.04	0.37	2.3	46	0.09	2.2	48
CIAT-Quilichao (residual effect+F)	10/03/82	4.2	7.4	6.5	1.00	0.33	0.22	4.6	75	0.22	3.7	-
CIAT-Quilichao (residual effect-F)	10/03/82	4.1	7.8	3.5	0.92	0.30	0.21	4.8	77	0.14	1.2	-
Carimagua-Agronomy (virgin soil)		4.3	5.0	3.0	0.50	0.30	0.08	3.5	80	-	-	-
Carimagua-Yopare (virgin soil)		5.0	2.6	1.3	0.22	0.06	0.04	2.1	87	-	1.4	-

All four experiments had four treatments and four replications. Each plot had 132 plants and every month either two or six plants were harvested. The plants were separated into leaf blades, petioles and stems of the 1/3 upper, middle and lower part of the plant, as well as the fibrous and tuberous roots. The 11 plants parts were analyzed for macro, secondary and micronutrients. In addition, soil samples were taken every month to follow changes in the soil fertility parameters.

1. First experiment on nutrient absorption and distribution in Quilichao in 1978/79

This experiment had four treatments, i.e. two varieties (MCol 22 and MMex 59), and with or without fertilization. All plots received 500 kg/ha of dolomitic lime, while the fertilized plots received 1000 kg/ha of 10-30-10 fertilizer, 20 kg S/ha as elemental sulfur, 10 kg Zn/ha as $ZnSO_4.7\ H_2O$ and 1 kg B/ha as Borax, all broadcast and incorporated into the soil before planting. Cassava stakes were planted at 80 x 80 cm for a population of 15,625 plants/ha. Each month two plants were harvested in each plot.

a. Dry matter production and distribution

Figure 3 shows the rainfall distribution as well as the DM accumulation in the roots and the whole plant for each variety and with or without fertilizers during the 12 month growth cycle. Plant growth was slow during the first two months, but DM accumulated rapidly and at a fairly constant rate between 2 and 12 MAP for MCol 22, but was more erratic in fertilized MMex 59 and actually declined in the unfertilized plots during the last four months of the growth cycle. MMex 59 had a greater top growth while MCol 22 had a higher root yield and a more marked response to fertilization. **Figure 4** shows the distribution of DM between roots, stems, leaves and petioles in unfertilized plots during the 12-month growth cycle of MMex 59 and MCol 22, while **Table 3** shows the data for fertilized and unfertilized MCol 22. In both varieties roots became the dominant sink for DM after the third month, followed by stems, leaves and petioles. DM in leaf blades and petioles decreased after the 4^{th} month due to the onset of the dry season and leaf fall being in excess of new leaf production. Fertilization of MCol 22 increased total DM production at time of harvest by 65% and DM accumulation in roots by 71%. **Table 4** shows the DM content of the different plant parts of fertilized and unfertilized MCol 22 during the growth cycle. The DM content of all plant parts tended to increase with the age of the plant, particularly in the case of roots, but only marginally in the case of leaf blades and petioles. The effect of fertilization on DM content was not consistent. At time of

harvest at 12 MAP, the fresh root yield of MCol 22 was 52.1 t/ha for the fertilized plants and 34.0 t/ha for the unfertilized plants, an increase of 53% due to fertilization.

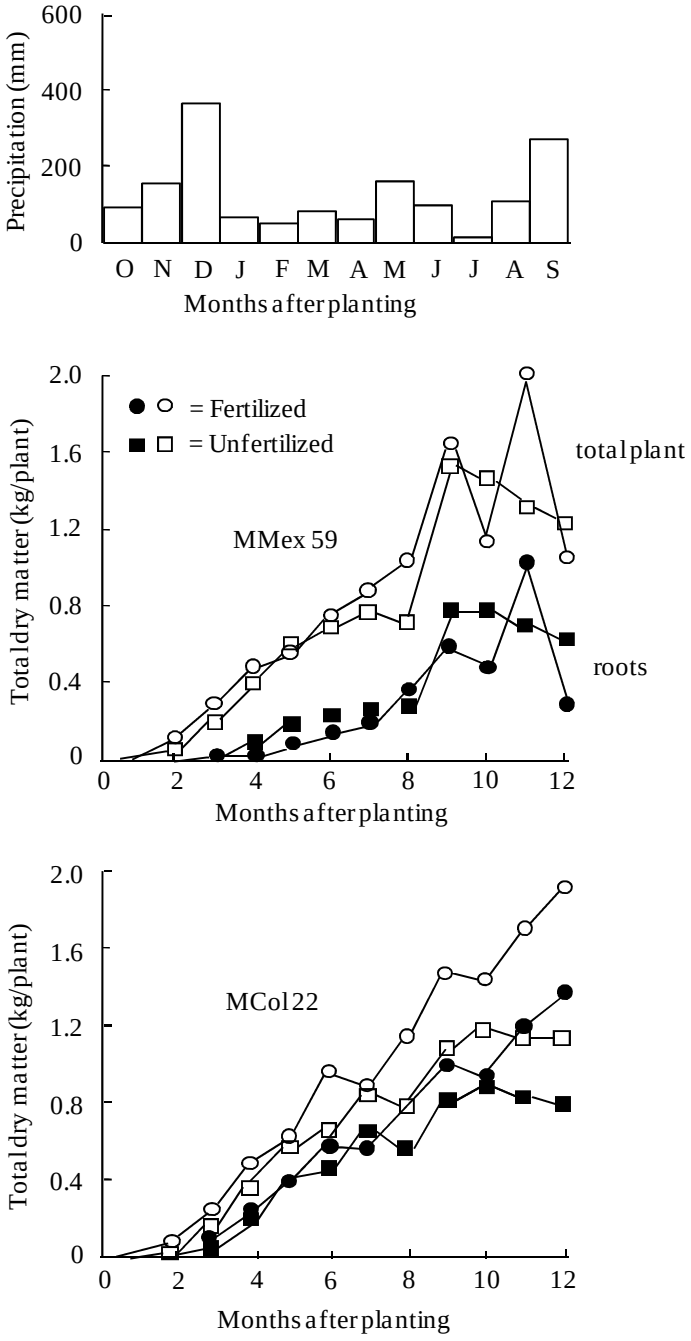

Figure 3. Monthly precipitation and accumulative dry matter production of total plant and of roots of cv. MMex 59 and MCol 22 grown with and without fertilizers during a 12-month growth cycle in Quilichao in 1978/79.
Source: *Howeler and Cadavid, 1983.*

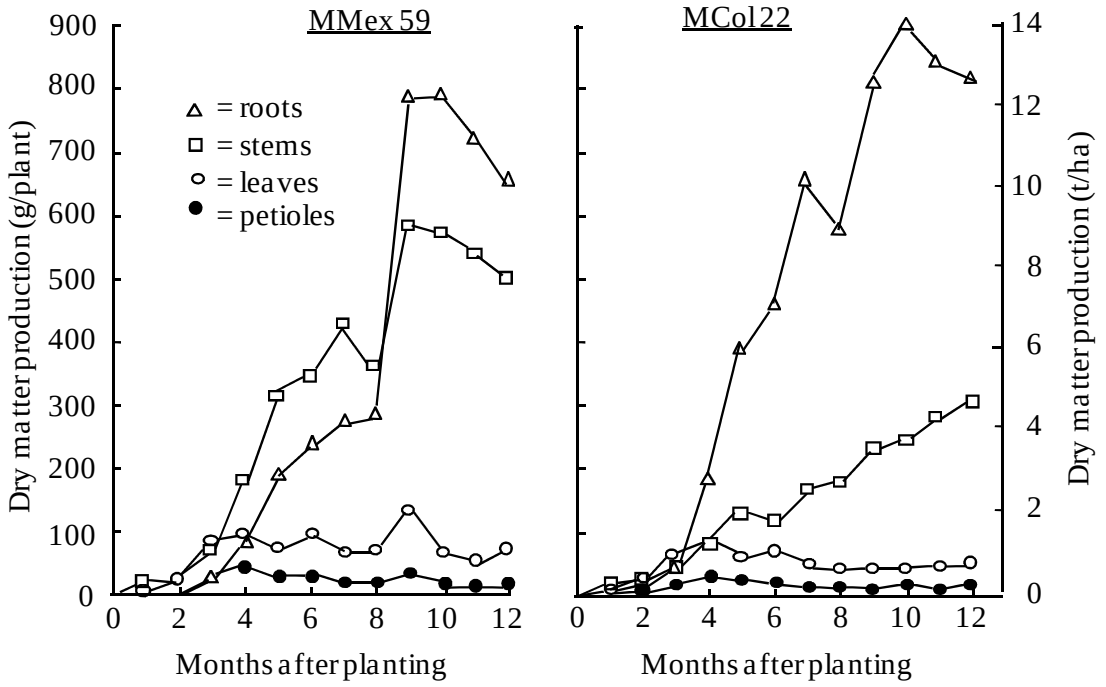

Figure 4. Distribution of dry matter among roots, stems, leaves and petioles of cassava, MMex 59 and MCol 22, during a 12-month growth cycle without applied fertilizers in Quilichao in 1978/79.
Source: *Howeler and Cadavid, 1983.*

Table 3. Dry matter distribution (g/plant) among various plant parts of fertilized and unfertilized MCol 22, during a 12-month growth cycle in Quilichao Colombia in 1978/79.

	Months after planting								
	1	2	3	4	5	6	8	10	12
Unfertilized									
-leaf blades	1.5	10.4	51.0	77.3	54.9	66.3	32.9	37.5	48.5
-petioles	0.2	1.9	13.6	25.6	18.4	17.1	5.9	6.9	7.5
-stems	13.2	16.0	37.1	79.4	128.3	114.1	173.8	239.5	300.1
-roots	0.2	1.4	43.7	178.0	380.1	448.5	572.4	896.3	811.3
Total	15.1	29.8	145.4	360.3	581.6	646.1	784.9	1180.1	1167.4
Fertilized									
-leaf blades	1.8	22.7	76.0	100.6	56.2	100.2	50.5	58.7	67.0
-petioles	0.2	4.9	21.5	38.2	19.0	27.4	8.6	12.1	11.5
-stems	14.1	29.1	58.9	125.2	182.1	269.1	302.7	428.6	459.9
-roots	0.1	7.1	80.5	229.6	360.0	571.9	782.6	942.4	1387.0
Total	16.2	63.8	236.9	493.7	617.3	968.6	1144.4	1441.8	1925.4

Table 4. Dry matter content (%) of leaf blades, petioles, stems and roots of fertilized and unfertilized MCol 22 during a 12-month growth cycle in Quilichao, Colombia in 1978/79.

	Months after planting								
	1	2	3	4	5	6	8	10	12
Fertilized									
-leaf blades	27.0	29.0	24.2	24.7	24.5	28.2	29.9	31.3	31.7
-petioles	9.7	12.6	11.8	16.3	18.5	16.1	15.6	20.2	18.1
-stems	19.4	14.6	11.9	20.0	20.8	21.5	24.7	28.5	23.9
-roots	8.7	12.4	15.3	22.8	30.4	31.1	35.5	37.1	41.6
Unfertilized									
-leaf blades	27.9	29.7	29.7	27.8	27.4	29.3	32.5	33.0	30.8
-petioles	10.0	15.3	13.2	18.7	18.6	16.6	18.4	21.3	16.0
-stems	19.3	15.8	13.1	20.1	22.3	18.8	23.2	27.1	25.5
-roots	8.0	13.6	13.4	25.1	29.7	31.4	35.5	39.4	37.3

b. Nutrient concentration in plant tissues

The nutrient concentrations in plants vary among the various tissues and at different positions in the plant, and also vary with time during the growth cycle. They are also affected by climatic conditions and by soil fertility or fertilization practices. **Table 5** shows the average nutrient concentrations in various plant parts of fertilized and unfertilized MCol 22 at 2-4 MAP when cassava plants normally have their highest growth rate and thus their greatest nutrient demand. For that reason, it is recommended that tissues be sampled for diagnostic purposes during this period.

With fertilizer application, plants were well supplied with all nutrients, resulting in high yields of 52.1 t/ha of fresh roots. Thus, the nutrient concentrations shown in **Table 5** for fertilized plants are considered near optimum, and those for upper leaf blades are above the critical levels reported in the literature (Howeler, 2002a) for youngest fully expanded leaf blades. Only the Mn concentration was unusually high due to high levels of this element in the soil.

The unfertilized plants can be considered low in P and B. Fertilization generally increased the tissue concentrations of N, P, B, Mn and Zn, increased that of K only in the leaf blades, had little effect on the concentrations of Ca, Mg and S, and decreased those of Fe (except in upper and middle leaf blades) and Cu. Calculating the percent change in nutrient concentrations due to fertilization in the different tissues for those elements in the fertilizers applied, it was found that nutrient concentrations in leaf blades were more affected by fertilization than those in petioles, stems and roots and that lower leaves were more affected than middle or upper leaves. However, lower leaves are more variable in nutrient content than upper leaves as they are of variable physiological age and therefore not very suitable for diagnostic purposes.

Table 5 shows that the N, P and S concentrations were highest in leaf blades, followed by stems, petioles and roots; they decreased markedly from the upper to the lower part of the plant. K, Ca and Mg concentrations, on the other hand, tended to be higher in the stem or petioles than in leaf blades, with the lowest concentrations again in the roots. The K concentration decreased markedly from the top to the bottom of the plant, but was

relatively high in the roots; Ca and Mg concentrations were highest in bottom leaves and petioles, but lower in bottom stems and especially in roots. Of the micro-nutrients, Fe and Mn tended to be high in the lower leaves and petioles. The rather high level of Fe in roots may have been caused by the roots being contaminated with soil.

Unlike Mn, the Fe concentration was found to be much lower in petioles than in the corresponding leaf blades. These large differences in nutrient concentrations between leaf blades and petioles, especially for N, P, K, S and Fe makes it imperative to sample leaf blades and petioles separately and not mix these two tissues in the same sample for diagnostic purposes.

Table 5. Concentration of nutrients in various plant parts of fertilized and unfertilized cassava. Data are average of samples of MCol 22 taken at 2, 3 and 4 months in Quilichao, Colombia in 1978/79.

	(%) N	(%) P	(%) K	(%) Ca	(%) Mg	(%) S	(ppm) B	(ppm) Cu	(ppm) Fe	(ppm) Mn	(ppm) Zn
Unfertilized											
Leaf blades											
-upper	5.06	0.31	1.72	0.59	0.31	0.30	6.4	12.7	154	288	79
-middle	4.08	0.21	1.53	0.85	0.36	0.29	7.1	11.5	243	356	75
-lower	3.50	0.16	1.38	1.21	0.49	0.25	7.3	9.1	422	444	75
Petioles											
-upper	2.23	0.19	3.26	1.00	0.41	0.08	8.6	11.0	105	496	77
-middle	2.07	0.09	2.45	1.40	0.49	0.03	6.8	7.2	55	934	118
-lower	1.39	0.08	2.02	1.98	0.66	0.02	8.2	6.1	192	1731	148
Stems											
-upper	3.24	0.27	3.44	0.96	0.42	0.23	8.3	20.1	148	321	73
-middle	3.55	0.23	2.08	1.21	0.48	0.26	7.6	29.7	122	374	110
-lower	1.27	0.12	0.69	0.83	0.35	0.10	6.5	24.6	247	132	54
Tuberous roots	1.35	0.13	1.58	0.26	0.13	0.03	4.9	13.1	509	162	59
Fertilized											
Leaf blades											
-upper	5.73	0.38	1.85	0.57	0.31	0.31	13.3	11.0	220	437	109
-middle	5.32	0.26	1.75	0.84	0.35	0.28	13.3	10.7	288	566	116
-lower	4.82	0.22	1.60	1.14	0.42	0.26	14.0	11.3	413	740	141
Petioles											
-upper	2.62	0.20	2.98	0.88	0.35	0.06	13.1	6.7	55	782	102
-middle	1.63	0.14	2.58	1.11	0.38	0.03	13.3	7.2	66	1060	150
-lower	1.58	0.11	2.28	1.61	0.53	0.02	14.5	6.5	104	1941	249
Stems											
-upper	3.11	0.31	3.10	0.88	0.37	0.17	13.5	14.8	116	451	109
-middle	2.79	0.35	2.35	1.06	0.44	0.13	11.2	22.0	128	505	157
-lower	1.34	0.20	0.80	0.71	0.35	0.06	6.6	18.2	195	169	133
Tuberous roots	1.36	0.17	1.51	0.19	0.11	0.03	6.5	8.8	306	107	58

The B concentration was more or less the same throughout the plant except in the stem, in which the concentration decreased from the upper to the lower part. Copper concentrations were highest in the stem followed by leaves, roots and petioles. The Zn concentration was more or less uniform throughout the plant, except that lower petioles had a higher concentration than upper petioles.

The change in nutrient concentration of selected tissues during the 12-month growth cycle is shown in **Figures 5** and **6**. Fertilized and unfertilized plants differed only about 10-20% in the concentration of most nutrients and showed a similar nutrient distribution, so the average of fertilized and unfertilized plants are shown. Only the B and Zn concentrations were markedly different between fertilized and unfertilized plants and the concentration in upper leaf blades is therefore shown separately for these two elements in **Figure 7**.

Except for Ca and Mg, the concentration of all nutrients increased in upper leaves during the first 2-3 months, after which it decreased. This decrease was particularly marked for N, P, K, S, B, Cu and Zn, while the Fe and Mn concentrations varied little over time. Unlike the other nutrients, the Ca and Mg concentrations of upper leaves decreased during the first two months and then increased to a maximum at about 4-6 months. **Figure 7** shows that the Zn concentration in upper leaf blades followed the same pattern during the growth cycle for both fertilized and unfertilized plants, only with the former at a higher concentration than the latter. The B concentration of upper leaves in fertilized plants was at least twice as high as in unfertilized plants and showed more variation during the growth cycle. However, even in the B-fertilized plants the B concentration of upper leaves remained far below the critical level for B deficiency of 35 ppm in YFEL blades, as determined in nutrient solution (Howeler *et al*., 1982). But, similarly low concentrations of B in upper leaves were reported in an earlier trial in Quilichao, which produced high yields of MCol 1684 of 53 to 60 t/ha irrespective of levels of B fertilization (CIAT, 1980).

Figures 5 and **6** show that nutrient concentrations in upper petioles generally followed the same pattern as that of leaf blades, except in the case of Mn in which the petiole concentration increased dramatically during the first six months and remained high until the 12^{th} month.

Nutrient concentrations in upper stem tissue generally decreased markedly with time, especially during the first six months. For this reason the upper stem is not a suitable tissue for diagnostic purposes. Instead, it is recommended to sample leaf blades as an indicator tissue, because of the relative constancy of their nutrient concentration over time and the less destructive nature of the sampling.

The nutrient concentration of roots also consistently decreased over time as roots accumulate starch and thus diluted the nutrient content. This was most marked for Fe, Mn, Cu and Zn, but was much less the case for P and Ca.

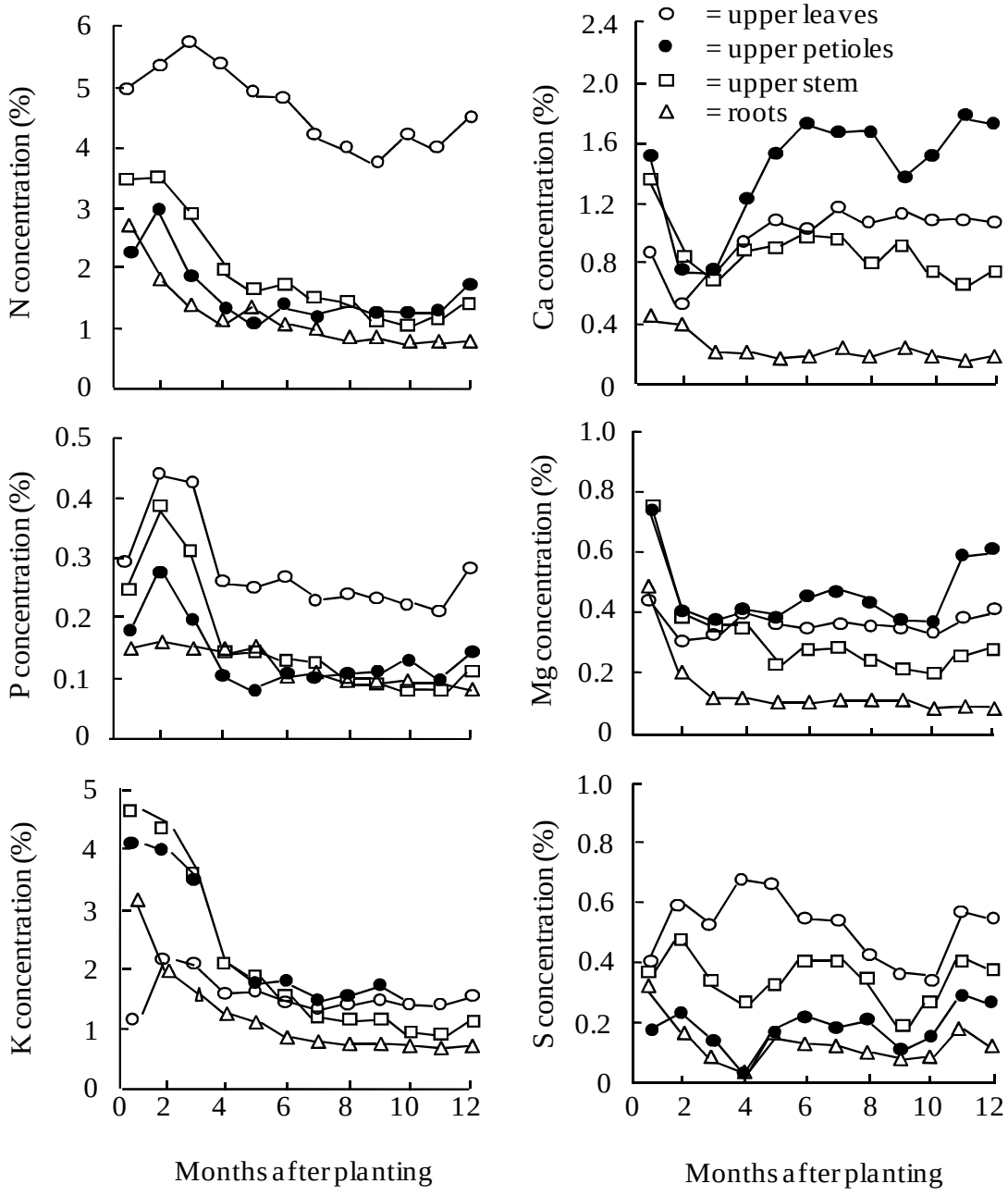

Figure 5. Concentration of macronutrients in upper leaves, petioles and stem as well as in the roots during a 12-month growth cycle. Data are the average of fertilized and unfertilized plants of MCol 22 and MMex 59
Source: *Howeler and Cadavid, 1983.*

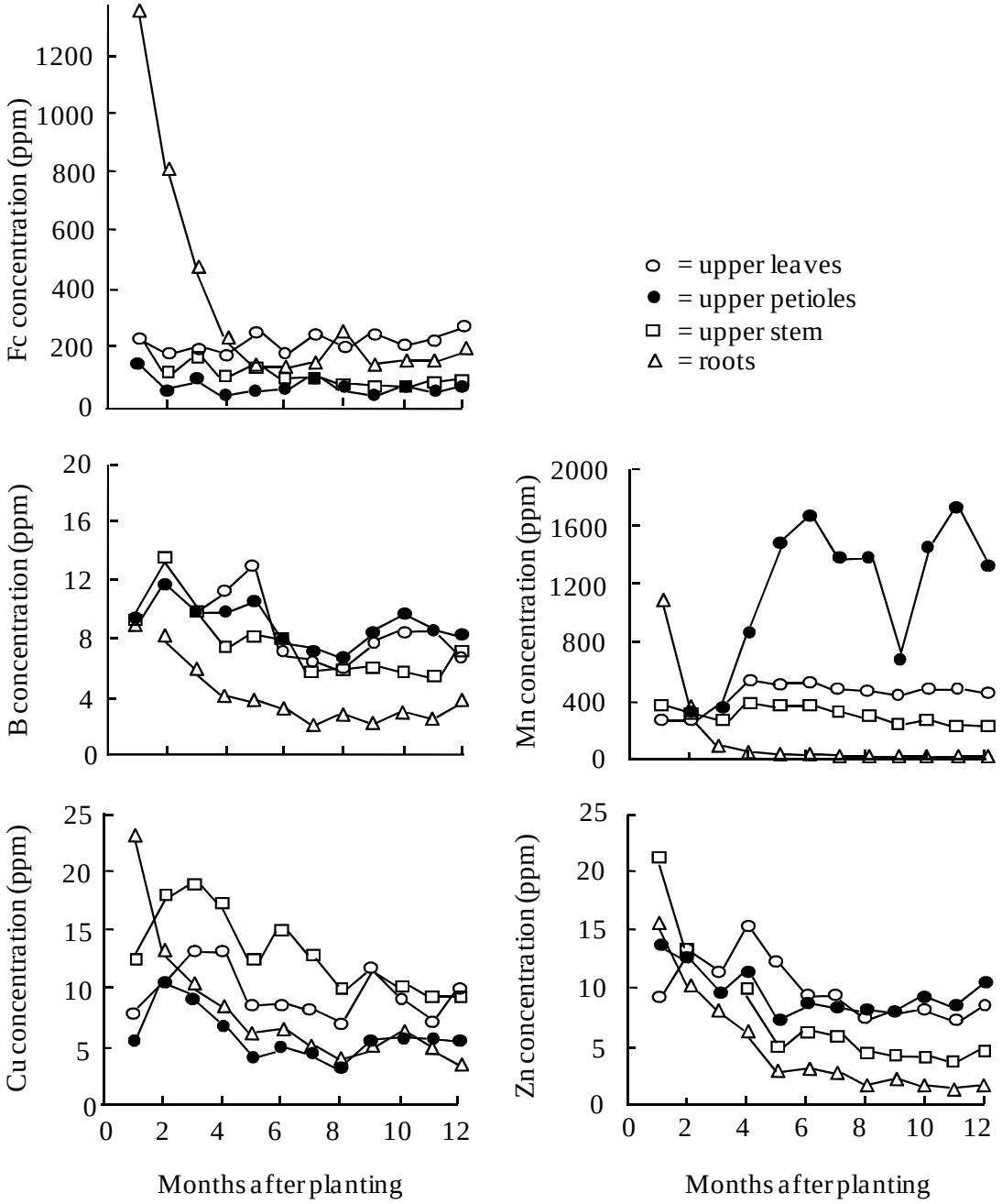

Figure 6. Concentration of micronutrients in upper leaves, petioles and stems as well as in the roots during a 12-month growth cycle in Quilichao in 1978/79. Data are the average of fertilized and unfertilized plants of MCol 22 and MMex 59.
Source: *Howeler and Cadavid, 1983.*

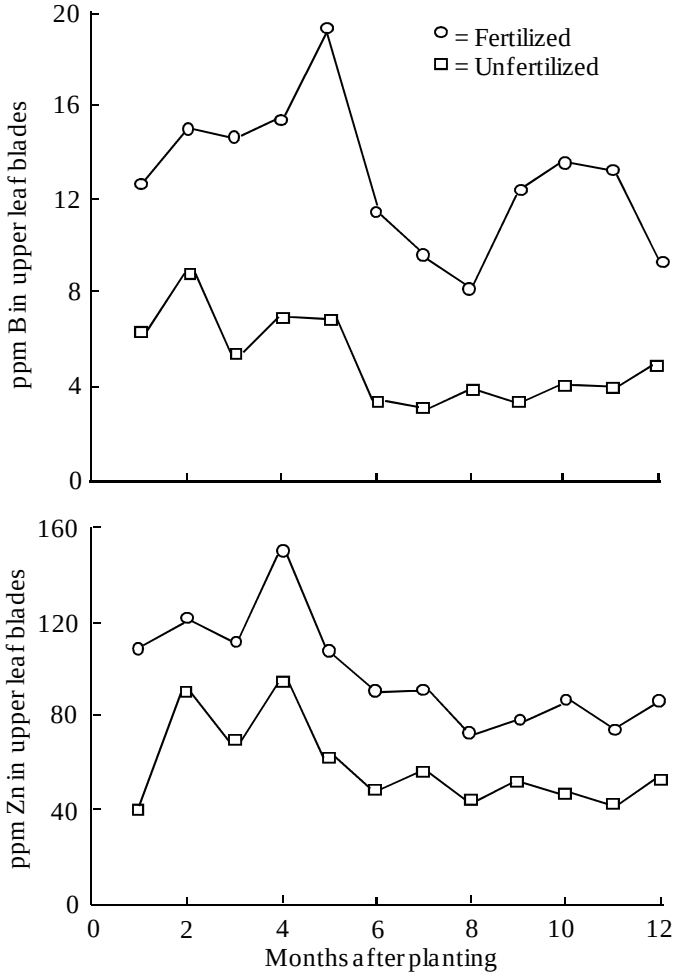

Figure 7. Concentration of B and Zn in upper leaf blades of fertilized and unfertilized cassava during a 12-month growth cycle. Data are averages for MCol 22 and MMex 59. ***Source:*** *Howeler and Cadavid, 1983.*

c. Nutrient uptake and distribution

Table 6 shows the total accumulation of DM and nutrients by fertilized and unfertilized MCol 22 during the growth cycle. Fertilized plants absorbed more nutrients than unfertilized plants, even of those elements not applied in the fertilizer, presumably because of a better root system and more dry matter production. Fertilization markedly increased the uptake of Zn, P and B while the increase in N and K absorption was essentially due only to an increase in dry matter production, except in the first two months of growth when the fertilizer also increased the N and K concentration in the plant. The application of fertilizers (including S) increased the uptake of S but below the increase in DM production, indicating that the S concentration in fertilized plants was overall lower than in unfertilized plants.

Table 6. Total dry matter (g/plant) and nutrient contents (mg/plant) of fertilized and unfertilized MCol 22 during a 12-month growth cycle in Quilichao, Colombia, in 1978/79.

		Months after planting								
		1	2	3	4	5	6	8	10	12
DM	Fertilized	16	64	237	494	617	969	1144	1442	1925
	Unfertilized	15	30	145	360	581	646	785	1180	1167
N	Fertilized	212	1913	6825	9956	10353	15051	14102	18292	20196
	Unfertilized	156	752	3428	6903	10285	9674	10179	8695	13410
P	Fertilized	42	164	611	811	1087	1372	1489	1582	2345
	Unfertilized	25	52	257	424	702	626	746	803	1141
K	Fertilized	96	834	4127	7012	7611	9176	9322	11130	15231
	Unfertilized	76	324	2443	5072	7153	6552	6557	8676	9216
Ca	Fertilized	236	490	1393	2501	2265	3895	3475	4536	4953
	Unfertilized	214	247	908	1977	2570	2809	2844	4369	4517
Mg	Fertilized	106	224	614	1184	946	1393	1512	1594	2071
	Unfertilized	96	115	388	896	936	978	1118	1396	1596
S	Fertilized	18	88	280	436	643	841	861	715	1389
	Unfertilized	18	43	191	395	636	693	572	752	1082
B	Fertilized	0.11	0.64	2.25	4.14	4.23	4.67	5.11	6.15	8.15
	Unfertilized	0.09	0.25	0.71	1.59	2.24	2.23	2.40	4.13	5.45
Cu	Fertilized	0.42	0.88	2.85	5.60	4.67	7.19	5.26	10.56	11.24
	Unfertilized	0.70	0.64	2.25	4.20	5.15	5.31	4.97	8.79	7.06
Fe	Fertilized	3.1	12.2	64.2	70.5	63.8	120.8	228.5	183.1	297.1
	Unfertilized	3.0	7.0	43.1	46.8	59.4	57.0	175.0	193.7	226.0
Mn	Fertilized	1.5	20.9	79.2	171.5	128.9	223.4	135.2	195.2	182.1
	Unfertilized	1.5	6.4	33.3	94.5	102.1	108.3	75.5	106.0	110.4
Zn	Fertilized	4.6	8.3	22.4	44.6	29.0	42.8	36.2	45.6	49.2
	Unfertilized	0.5	3.4	8.3	21.8	16.3	17.4	14.7	17.3	19.7

Nutrients accumulated during the entire growth cycle, the maximum increase in accumulation occurring between 2 and 4 months. This period corresponds to maximum DM accumulation. After about six months the uptake rate of most nutrients decreased and became almost nil in the case of Mn and Zn. However, all other nutrients continued to be absorbed by the plant throughout the 12 month period.

Table 7 shows that N, P, and K accumulated mainly in the roots. Consequently, much of these nutrients are removed from the field with the harvested product. Cu, Fe and B also accumulated mainly in the roots, while Ca, Mg, Mn, S and Zn accumulated principally in the stem. At harvest time the nutrient content of leaves and petioles was

seldom more than 10 or 15% of that in the total plant, as these nutrients had either been recycled to other plant parts or had returned to the soil during the leaf fall.

Table 8 shows that for most nutrients the accumulation in the roots accounted for less than 50% of the total, indicating that the incorporation of stems and leaves into the soil would greatly diminish total nutrient export and thus the requirement for fertilizer application. However, nearly two thirds of the total amount of absorbed K accumulated in the roots and would thus be removed at harvest, corresponding to 162 kg K/ha. During the last 6 months of the growth cycle N was rather evenly distributed between roots and tops.

Table 7. Dry matter (g/plant) and nutrient content (mg/per plant) in various parts of fertilized MCol 22, during a 12-month growth cycle in Quilicho, Colombia, in 1978/79.

		Months after planting								
		1	2	3	4	5	6	8	10	12
DM	Leaves	1.8	22.7	76.0	100.6	56.2	100.2	50.5	58.7	67.0
	Petioles	0.2	4.9	21.5	38.2	19.0	27.4	8.6	12.1	11.5
	Stems	14.1	29.1	58.9	125.2	182.1	269.1	302.7	428.6	459.9
	Roots	0.1	7.1	80.5	229.6	360.0	571.9	782.6	942.4	1387.0
	Total	16.2	63.8	236.9	493.7	617.3	968.6	1144.4	1441.8	1925.4
N	Leaves	89	1231	4230	5300	2703	4877	2206	2702	3350
	Petioles	6	134	368	485	202	378	144	182	207
	Stems	117	422	1146	1919	3022	4191	4707	5984	6930
	Roots	-	125	1078	2250	4428	5605	7043	9424	9709
	Total	212	1912	6824	9954	10355	15051	14100	18292	20196
P	Leaves	5	71	267	227	137	288	136	147	174
	Petioles	-	10	35	34	16	31	10	20	18
	Stems	37	71	157	205	358	422	482	378	766
	Roots	-	11	153	344	576	629	861	1036	1387
	Total	42	163	612	810	1087	1370	1489	1581	2345
K	Leaves	24	337	1408	1716	507	1564	712	817	945
	Petioles	9	161	598	744	347	561	159	201	207
	Stems	58	213	872	1681	2581	2588	2817	3233	3676
	Roots	5	123	1248	2870	4176	4463	5635	6879	10402
	Total	96	834	4126	7011	7611	9176	9323	11130	15230
Ca	Leaves	15	157	583	924	525	857	424	452	435
	Petioles	4	68	212	393	248	420	125	165	186
	Stems	216	244	485	864	1061	1704	1986	2412	3083
	Roots	1	20	113	321	432	915	939	1508	1248
	Total	236	489	1393	2502	2266	3895	3474	4537	4952
Mg	Leaves	9	67	248	411	166	276	146	146	174
	Petioles	2	23	77	142	68	130	32	41	56
	Stems	93	125	216	401	424	586	707	746	1147
	Roots	-	9	72	230	288	400	626	660	693
	Total	104	224	613	1184	946	1392	1511	1593	2070

Table 7. Continued

		Months after planting								
		1	2	3	4	5	6	8	10	12
S	Leaves	2	61	203	335	185	256	101	88	241
	Petioles	-	4	5	-	14	30	7	7	14
	Stems	15	19	63	101	227	383	360	337	578
	Roots	-	5	8	-	216	171	391	283	555
	Total	17	89	279	436	642	840	859	715	1388
B	Leaves	0.02	0.29	0.98	1.44	1.02	1.00	0.40	0.73	0.62
	Petioles	-	0.0	0.29	0.52	0.32	0.32	0.08	0.17	0.13
	Stems	0.09	0.22	0.48	0.99	1.31	1.89	2.07	2.42	3.52
	Roots	-	0.06	0.50	1.19	1.58	1.37	2.50	2.83	3.88
	Total	0.11	0.64	2.25	4.14	4.23	4.67	5.11	6.15	8.15
Cu	Leaves	0.02	0.20	0.89	1.22	0.44	0.89	0.37	0.58	0.71
	Petioles	-	0.03	0.18	0.22	0.07	0.13	0.03	0.08	0.07
	Stems	0.40	0.59	1.09	2.14	2.04	2.74	2.36	3.87	3.80
	Roots	-	-	0.69	2.02	2.12	3.43	2.50	6.03	6.66
	Total	0.42	0.82	2.85	5.60	4.67	7.19	5.26	10.56	11.24
Fe	Leaves	0.6	4.3	32.4	22.3	14.6	20.6	10.2	12.9	11.7
	Petioles	-	0.3	2.1	1.7	1.1	1.4	0.5	1.0	0.8
	Stems	2.4	3.2	15.0	17.8	15.7	38.7	52.7	43.8	141.7
	Roots	-	4.3	14.6	28.7	32.4	60.0	165.1	125.3	142.9
	Total	3.0	12.1	64.1	70.5	63.8	120.7	228.5	183.0	297.1
Mn	Leaves	0.4	10.7	38.3	66.6	27.5	67.5	25.2	31.5	26.4
	Petioles	0.1	5.2	19.5	52.4	37.8	64.6	15.5	21.2	21.0
	Stems	0.8	3.4	16.9	40.7	46.3	67.8	74.1	106.7	108.4
	Roots	0.2	1.5	4.5	11.7	17.3	23.4	20.3	35.8	26.3
	Total	1.5	20.8	79.2	171.4	128.9	223.3	135.1	195.2	182.1
Zn	Leaves	0.19	2.75	7.37	13.56	5.39	8.23	3.54	4.29	4.76
	Petioles	0.04	0.86	3.09	5.15	1.64	2.61	0.86	1.33	1.50
	Stems	4.34	4.17	8.19	15.06	12.93	17.67	17.74	23.94	26.27
	Roots	0.02	0.52	3.78	10.79	9.00	14.30	14.09	16.02	16.64
	Total	4.59	8.30	22.43	44.56	28.96	42.81	36.23	45.58	49.17

Although much of the absorbed N would return to the soil with leaves and stems, still about 9.7 g per plant or 152 kg/ha of N was removed in the final root harvest. While the removal of N in the root harvest may be nearly as high as that of K, it appears less serious as the soil has a high N supplying power, or the plant has the ability to somehow fix N. In any case, in a long-term fertility trial, after three consecutive crops of cassava, there was no significant response to N application, while the N content of leaves remained high even in the absence of applied N. On the other hand, the annual application of K markedly increased the K contents of leaves and nearly doubled the yields. Roots also contain a considerable fraction of absorbed P, but exhaustion or “mining” of P from the soil is less likely than that of K since generally only a small amount of P applied is absorbed by the plant and the rest accumulates in the soil through P fixation. In this experiment only 16% of applied P was removed in the root harvest of MCol 22.

Table 8. Percent of total nutrients present in the roots of fertilized MCol 22 during the growth cycle. Numbers in parenthesis indicate kg/ha of nutrients removed in the final root harvest.

	Months after planting								
Nutrient	1	2	3	4	5	6	8	10	12
N	-	7	16	23	43	37	50	52	48 (152)
P	-	7	25	42	53	46	58	65	59 (22)
K	5	15	30	41	55	49	60	62	68 (162)
Ca	-	4	8	13	19	23	27	33	25 (20)
Mg	-	4	12	19	30	29	41	41	23 (11)
S	-	6	3	-	34	20	45	40	40 (9)
B	-	1	22	29	37	29	49	46	48 (0.06)
Cu	-	-	24	36	45	48	48	57	59 (0.10)
Fe	-	36	23	41	51	50	72	68	48 (2.2)
Mn	13	7	6	7	13	10	15	18	14 (0.41)
Zn	-	6	17	24	31	33	39	35	34 (0.26)

d. Changes in soil characteristics

Analyses of soil samples taken monthly between plants in the ridge (**Table 9**) indicate that only minor changes occurred in pH and exchangeable Al, Ca, and Mg, and that the absence or presence of fertilizers had no major effect on these soil characteristics. There were no significant differences between samples taken at 20 and 40 cm from the plants. Soil pH fluctuated between 3.7 and 4.2 with an average of 4.0; exchangeable Al fluctuated from 1.8 to 3.8 meq/100 g with an average of 3.1 meq/100 g, with a slight tendency to increase from 2.8 to 3.4 meq/100 g during the growth cycle; exchangeable Ca remained rather constant with an average of 2.01 meq/100 g, while exchangeable Mg decreased slightly from 1.42 to 1.0 meq/100 g.

Table 9. Change in soil chemical characterictics during a 12-month growth cycle of fertilized and unfertilized plants of MCol 22 in Quilichoa, Colombia in 1978/99.

	Months after planting								
	1	2	3	4	5	6	8	10	12
pH									
-fertilized	4.10	4.12	3.82	4.05	4.12	4.00	4.00	4.00	3.80
-unfertilized	4.02	3.97	3.90	4.17	4.22	4.02	4.02	4.07	3.75
Avail-P (ppm)									
-fertilized	20.4	34.1	22.8	36.0	55.6	33.9	26.5	38.7	26.0
-unfertilized	4.1	3.6	4.0	2.9	4.1	4.0	3.4	4.2	4.3
Exch. K (meq/100 g)									
-fertilized	0.60	0.49	0.40	0.38	0.36	0.37	0.32	0.28	0.29
-unfertilized	0.37	0.43	0.30	0.27	0.20	0.26	0.25	0.26	0.24
Exch. Ca (meq/100 g)									
-fertilized	2.55	2.14	2.10	2.26	2.43	2.61	1.82	1.84	2.16
-unfertilized	1.89	1.77	1.75	1.89	1.94	2.01	1.67	1.69	1.66
Exch. Mg (meq/100 g)									
-fertilized	1.59	1.45	1.18	1.17	1.18	1.17	0.94	0.98	0.96
-unfertilized	1.26	1.36	1.02	1.06	1.06	1.08	0.91	0.94	0.94
Exch. Al (meq/100 g)									
-fertilized	2.35	1.82	3.57	2.95	3.00	2.65	3.05	3.67	3.35
-unfertilized	2.82	2.50	3.80	3.07	3.22	2.75	3.20	3.50	3.37
Al-saturation (%**)**									
-fertilized	33	31	49	44	43	39	50	54	50
-unfertilized	44	41	55	49	50	45	53	55	54

Figure 8 shows the change in available P and exchangeable K during the crop cycle, with and without fertilization. Without fertilization, soil P (Bray II) remained rather constant with an average of 4.1 ppm. However, with fertilization soil P fluctuated greatly between 20 and 55 ppm with an average value of 33 ppm. The high values appear to be associated with dry periods, while the low values at 3, 8 and 12 months corresponded with rainfall peaks. Sampling during periods of severe drought or excessive rainfall are therefore not recommended. From **Figure 8A** it is clear that soil P did not change appreciably by plant uptake since P absorption was relatively low compared with soil reserves (especially organic P in this soil).

However, the situation is quite different for exchangeable K. **Figure 8B** shows that without fertilization soil-K decreased from about 0.40 to 0.24 meq/100 g, while with fertilization it decreased from 0.60 to 0.29 meq/100 g. Thus, in both fertilized and unfertilized plots soil-K decreased significantly owing to plant uptake, and even if all stems and leaves were to be returned to the field, there would be a considerable net loss of K from the soil. If at harvest time each plant had accumulated 6 g K in the roots (as in unfertilized MCol 22), the harvest of these roots would correspond with a loss of about 100 kg K/ha or 0.13 meq/100 g in the top 20 cm of soil. According to data reported in the literature (Howeler, 1981), the harvest of cassava roots removes on average 4.1 kg K per ton of fresh roots. With a root harvest of 25 t/ha, this also corresponds to about 100 kg K/ha. Besides plant uptake, soil K is also lost by leaching and erosion; a decrease of about 0.16 meq K/100 g, as observed in this experiment, can thus be largely accounted for by crop removal. Even though crop response to applied K may be small during the first year, long-term

fertility trials with cassava have shown that without adequate K fertilization yields decline and the soil becomes impoverished in this element (CIAT, 1981; Chan, 1980; Den Doop, 1937). A long-term fertility trial at the same location (CIAT, 1982) has shown that, after three consecutive crop cycles of cassava, levels of exchangeable K had dropped from 0.21 to 0.09 without applied K, and to 0.14 meq/100 g with the annual application of 125 kg K/ha. Only the annual application of 250 kg K/ha could maintain and slightly increase the soil K level to 0.29 meq/100 g.

Thus, it appears imperative for sustained high yields of cassava on soils of low K-supplying power to apply about 100-150 kg K/ha, in order to offset losses by crop removal. Phosphorus fertilization, however, should be based not on crop removal but on crop response, applying the minimum amount for maximum economic yield.

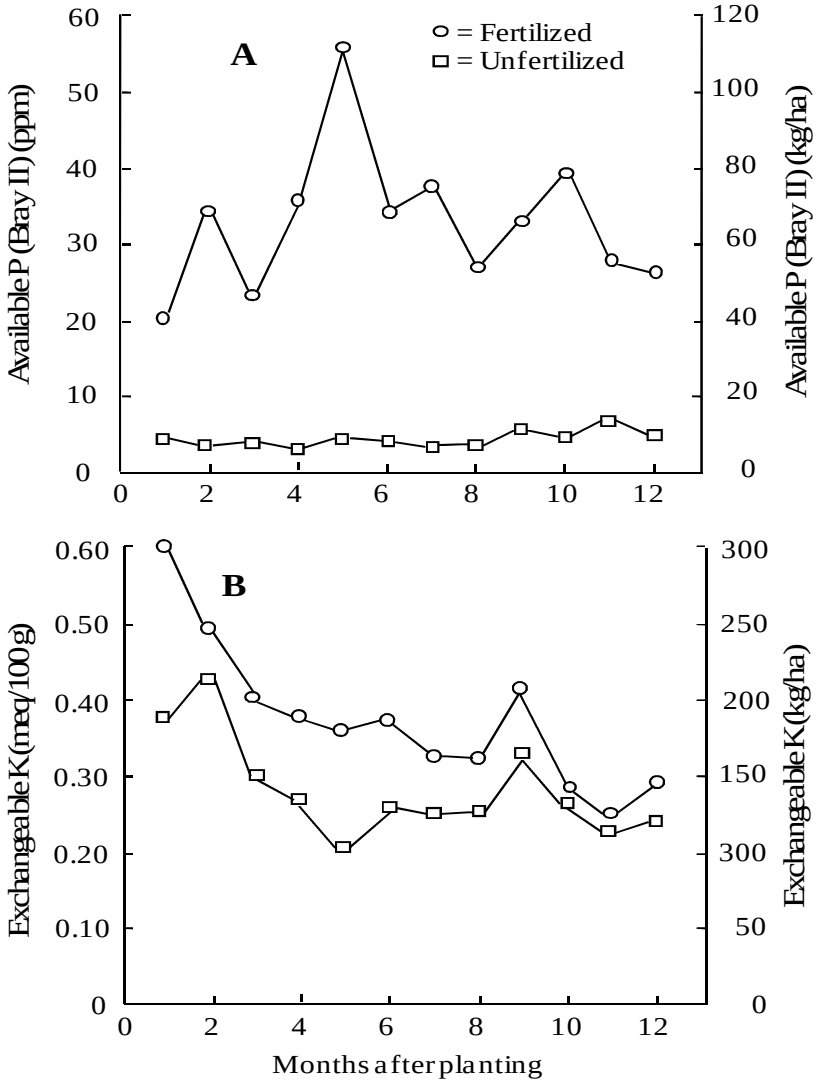

Figure 8. Change in available P and exchangeable K content of soil during a 12-month growth cycle of fertilized and unfertilized cassava in Quilichao, Colombia in 1978/79. ***Source:*** *Howeler and Cadavid, 1983.*

2. Second experiment on nutrient absorption and distribution in Quilichao in 1982/83

The experiment was conducted in the same field as the previous trial, conducted four years earlier in 1978/79. The experiment had two treatments, i.e. with and without fertilizers applied before planting, and four replications. Each plot had 12 subplots of six plants which were always surrounded by one internal and two external border rows. These six adjacent plants were harvested every month in each plot. To be able to harvest six instead of only two plants per plot, each treatment was located on two adjacent plots used in the previous experiment, two replications with and two without the residual effect of the previous fertilizer treatments. **Table 2** shows that four years after their application there was only a residual effect of the previous fertilization in terms of higher levels of P, B and Zn.

For the new experiment, all plots received another 500 kg/ha of dolomitic lime, while the plots with the fertilizer treatment received 1000 kg/ha of 10-30-10 and 1 kg B/ha as Resorita 65, both broadcast and incorporated before planting stakes of MCol 22 in March, 1982. The six plants harvested each month were again separated into the upper, middle and lower leaf blades, petioles and stem, while roots were further separated into fibrous and tuberous roots; in addition, the leaves fallen during the previous month were collected and separated into fallen leaf blades and petioles. These 13 samples were weighed fresh, washed and oven-dried to determine their dry weights and nutrient concentrations. Soil samples were also taken monthly in each treatment.

a. Dry matter production and distribution

Figure 9 shows the total DM produced, including fallen leaves by fertilized and unfertilized MCol 22 during a 12 month growth cycle in Quilichao, while **Figure 10** shows the distribution of DM among roots, tops and fallen leaves. DM accumulated at a fairly constant rate between the 2nd and 8th month, after which it slowed down and actually decreased slightly during the 11th and 12th month, probably due to the effect of the dry season in Feb and March. At time of harvest at 12 MAP, fertilized plants had accumulated 865 g DM/plant, of which 561 g were in the roots and 135 g in fallen leaves, while unfertilized plants accumulated a total of 631 g/plant of which 439 g in the roots and 94 g in fallen leaves. **Table 10** shows that DM accumulation in leaf blades and petioles increased rapidly during the 2nd and 3rd month, slowed down markedly during the 4th to 6th month due to the dry season, increased rapidly again during the 7th and 8th month and decreased during the following four months due to leaf fall and the effect of the dry season. Leaf fall started in the 3rd month, increased during the 4th and 5th months, after which it decreased, especially during the final two months of the growth cycle. Fresh root yields were 22 t/ha for fertilized and 17 t/ha for unfertilized plants, considerably lower than those obtained in the previous trial in 1978/79, probably due to a severe dry season in July/Aug 1982 and excess rain from Sept to Dec.

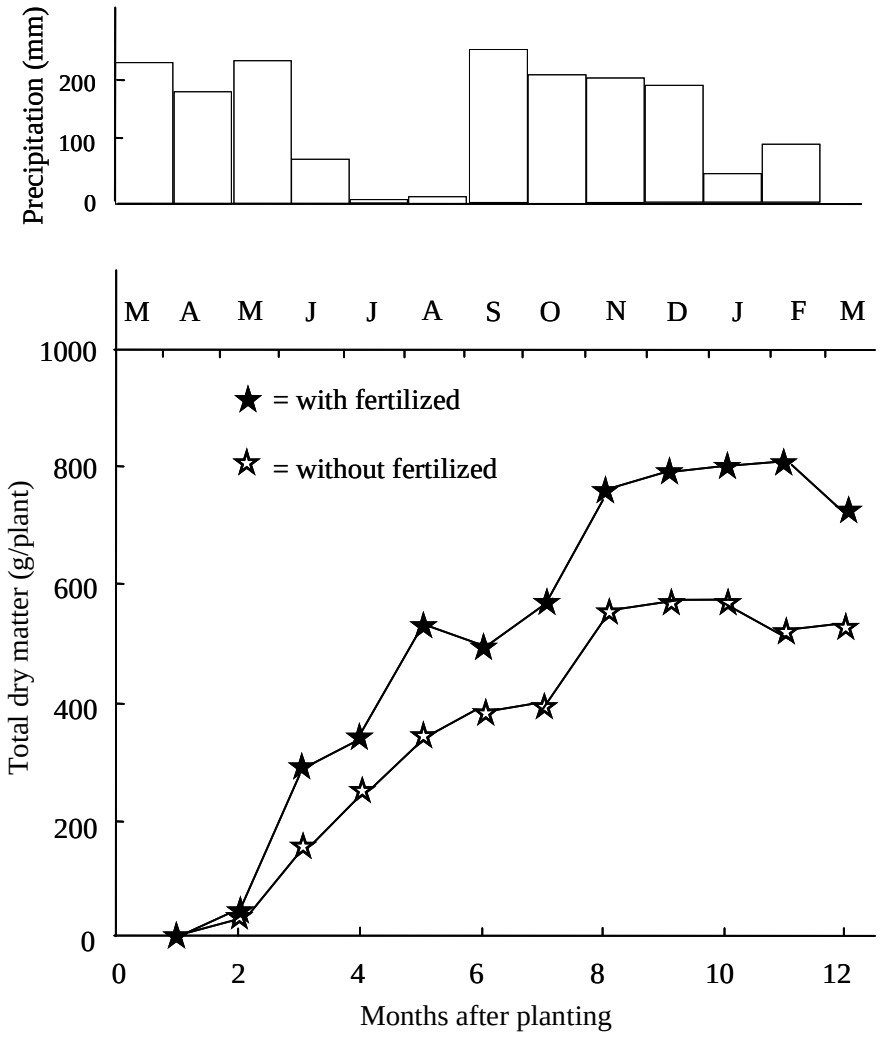

Figure 9. Monthly precipitation and accumulative total dry matter in fertilized and unfertilized cassava, MCol 22, during a 12-month growth cycle in Quilichao in 1982/83.

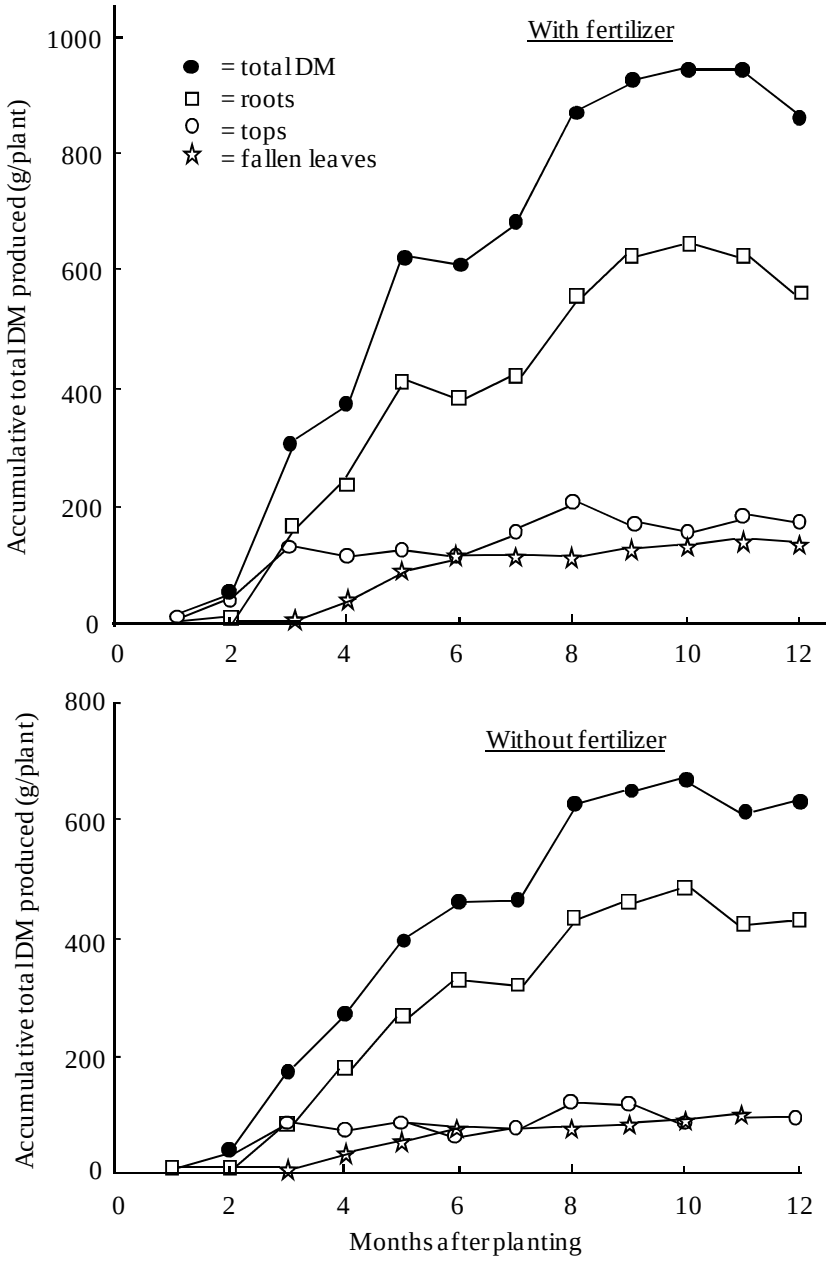

Figure 10. Accumulative total dry matter produced, and its distribution between tops, roots and fallen leaves of fertilized (top graph) and non-fertilized (bottom) cassava, MCol 22, during a 12-month growth cycle in Quilichao in 1982/83.

Table 10. Dry matter distribution (g/plant) among various plant parts of fertilized and unfertilized MCol 22 during a 12 month growth cycle in Quilichao, Colombia, in 1982/83.

	Months after planting								
	1	2	3	4	5	6	8	10	12
Unfertilized									
-leaf blades	3.2	16.3	43.9	35.7	27.9	10.4	39.1	7.5	9.4
-petioles	0.6	3.9	9.6	9.4	4.9	2.4	7.1	0.9	1.3
-stems	0.9	6.7	22.7	28.1	47.9	49.4	71.3	81.3	87.9
-roots	0.5	6.6	82.3	174.2	263.6	323.1	434.2	489.8	438.6
-fallen leaves[1)]	-	-	3.2	20.4	51.9	75.3	78.6	92.5	93.6
Total	5.2	33.5	161.7	267.8	396.2	460.6	630.3	672.0	630.8
Fertilized									
-leaf blades	3.8	20.6	69.0	49.8	37.8	14.6	70.1	10.9	15.1
-petioles	0.9	5.6	14.7	13.4	10.7	3.2	12.0	1.5	2.1
-stems	1.0	8.9	43.0	48.2	78.4	95.2	126.4	145.8	151.1
-roots	0.3	11.9	165.9	231.9	410.4	384.3	552.6	649.1	561.2
-fallen leaves[1)]	-	-	8.8	31.1	83.1	112.7	116.6	133.5	135.0
Total	6.0	47.0	301.4	374.4	620.4	610.0	877.7	940.8	864.5

[1)] Cumulative DM in fallen leaves.

b. Nutrient concentrations in plant tissues

Table 11 shows the average nutrient concentration in various plant parts collected at 2, 3 and 4 months after planting, both for fertilized and unfertilized MCol 22. This is the period when plants normally have their fastest growth rate, and thus their greatest nutrient demands.

Table 11. Concentration of nutrients in various plant parts of fertilized and unfertilized cassava. Data are average of samples taken at 2, 3 and 4 months of MCol 22 in Quilichao, Colombia, in 1982/83.

	(%) N	(%) P	(%) K	(%) Ca	(%) Mg	(%) S	(ppm) B	(ppm) Cu	(ppm) Fe	(ppm) Mn	(ppm) Zn
Unfertilized											
Leaf blades											
-upper	4.87	0.35	1.59	0.80	0.31	0.37	13.0	11.3	220	303	89
-middle	4.76	0.27	1.51	1.01	0.36	0.35	12.1	9.9	253	298	101
-lower	3.83	0.21	1.40	1.31	0.43	0.31	15.0	9.7	443	297	109
Petioles											
-upper	1.61	0.16	2.30	1.47	0.39	0.11	12.3	6.9	146	596	81
-middle	1.38	0.12	1.78	1.70	0.47	0.09	11.3	4.8	86	903	135
-lower	1.10	0.10	1.29	1.85	0.55	0.10	14.0	4.8	254	1054	174
Stems											
-upper	2.81	0.31	2.46	1.44	0.41	0.32	16.3	17.8	138	321	80
-middle	2.32	0.20	1.44	1.30	0.44	0.34	11.0	20.9	114	312	111
-lower	1.86	0.15	0.96	0.94	0.38	0.22	9.3	15.4	132	180	89
Fibrous roots	1.72	0.14	1.62	0.59	0.30	0.35	19.3	125.3	11797	866	83
Tuberous roots	1.02	0.12	0.99	0.29	0.10	0.06	11.3	7.8	1760	81	45
Fallen leaf blades	2.44	0.11	0.63	1.52	0.43	0.20	13.5	10.8	2459	340	126
Fallen petioles	0.73	0.04	0.27	1.78	0.49	0.08	13.2	3.8	308	1417	187
Fertilized											
Leaf blades											
-upper	5.12	0.39	1.68	0.83	0.34	0.37	21.3	11.7	173	395	78
-middle	5.12	0.31	1.73	1.05	0.36	0.33	18.3	10.1	227	362	67
-lower	4.16	0.25	1.53	1.31	0.42	0.31	25.3	10.0	409	452	93
Petioles											
-upper	1.58	0.22	2.30	1.40	0.37	0.09	18.1	6.4	78	807	71
-middle	1.39	0.15	1.83	1.58	0.43	0.06	16.0	6.0	100	1028	95
-lower	1.33	0.13	1.49	1.92	0.53	0.06	21.0	5.6	149	1353	149
Stems											
-upper	2.69	0.37	2.55	1.34	0.36	0.23	18.1	16.7	114	419	72
-middle	2.23	0.36	1.88	1.29	0.40	0.22	17.3	19.9	94	386	98
-lower	1.89	0.25	1.24	0.91	0.32	0.14	11.0	17.0	133	231	65
Fibrous roots	1.68	0.17	1.81	0.57	0.25	0.23	19.7	65.6	9892	840	70
Tuberous roots	1.21	0.17	1.21	0.25	0.10	0.06	16.3	6.4	724	75	32
Fallen leaf blades	2.85	0.16	0.85	1.63	0.45	0.22	24.0	11.2	2184	498	88
Fallen petioles	0.81	0.06	0.43	1.87	0.43	0.05	18.5	4.5	272	1499	158

Nutrient concentrations in practically all plant parts were considerably lower than those obtained in 1978/79, both for the fertilized and unfertilized plants, but their distribution was very similar to that obtained in the earlier trial. For both the fertilized and unfertilized plants the nutrient concentrations in upper leaf blades were still above the critical levels as determined in YFEL blades at 3-4 MAP (Howeler, 2002a). While in the 1978/79 trial the Zn concentrations in all above-ground plant parts were considerably higher in fertilized than in unfertilized plants, this was not the case in the 1982/83 trial,

probably because in the latter trial no Zn was applied to the fertilized plants. But the P, B and Zn concentrations in unfertilized plants in this trial were higher than those in 1978/79, because of the residual effect of these elements from their application in the 1978/79 experiment.

As in 1978/79 the N, P and S concentrations were highest in the leaf blades, followed in stems and petioles; they decreased from the upper to the lower part of the plants. The K, Ca and Mg concentrations were higher in the stems and petioles than in the leaf blades with lowest levels again in the tuberous roots, especially for Ca and Mg. K concentrations decreased from the top to the bottom of the plant, but with a relatively high concentration in the tuberous roots, while the Ca and Mg concentrations increased from the top to the bottom of the plant in the leaf blades and petioles but not in the stems. The micronutrients also had similar patterns among the various plant parts as in the 1978/79 trial described above.

The change in concentration of N, P and K in leaf blades from the upper, middle and lower part of the plant, as well as of fallen leaves during the 12 month growth cycle is shown in **Figure 11**. The concentration of all three nutrients decreased during the first 3-4 months and then more or less stabilized. The concentration decreased from the upper to the lower leaves and was lowest in the fallen leaves, indicating that nutrients had translocated from older tissues to the younger tissues. Still, the nutrient concentrations in fallen leaves were substantial, especially N, which means that these nutrients are being recycled to the soil. The concentrations of S, B, Cu, Fe and Zn in leaf blades also decreased markedly during the first 3-4 months before stabilizing during the remainder of the growth cycle. In contrast, the concentration of Ca, Mg and Mn remained rather constant throughout the growth cycle. The nutrient concentration in tuberous roots decreased gradually during the growth cycle as roots filled with starch.

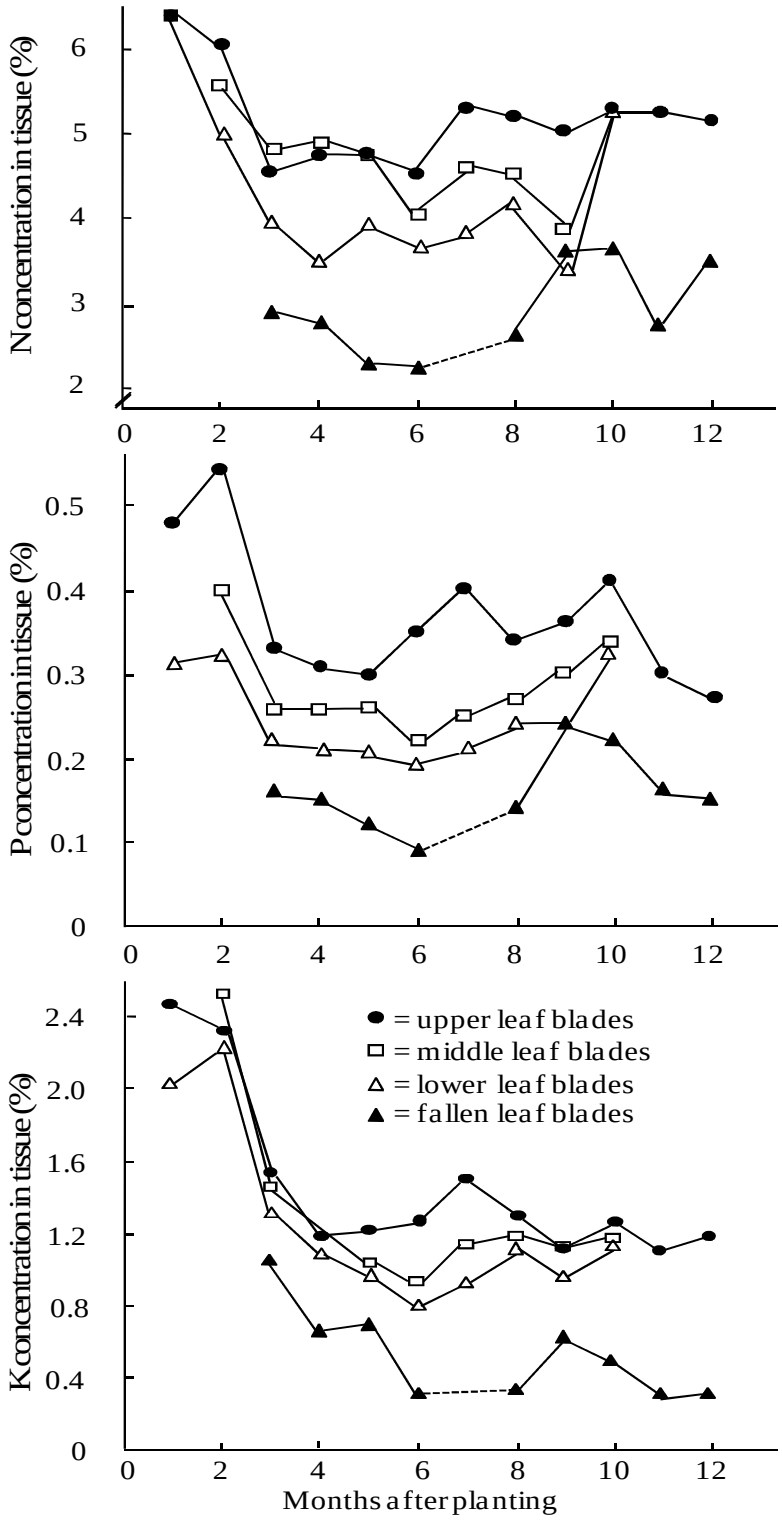

Figure 11. Concentration of N, P and K in leaf blades from the upper, middle and lower part of the plant as well as from fallen leaves of fertilized cassava, MCol 22, during a 12-month growth cycle in Quilichao in 1982/83.

c. Nutrient uptake and distribution

Table 12 shows the accumulation of DM and nutrients in fertilized and unfertilized plants of MCol 22 during the 12-month growth cycle, without inclusion of fallen leaves. Fertilization markedly increased the uptake of all nutrients, mainly due to increased root development and growth (**Figure 10**). Fertilization increased total DM production throughout the growth cycle about 42%, increased the average absorption of N by a similar 42%, P by 77%, K by 49% and B by 89%, indicating that fertilization did not increase substantially the concentration of N and K, but did increase that of P and B in most plant tissues.

Table 13 shows the dry matter and nutrient accumulation and distribution between plant tops, roots and fallen leaves during the growth cycle of fertilized MCol 22. About 43% of N, 61% of P and 63% of absorbed K was present in the roots during the three final months of the growth cycle. This contrasts with only 25% of Ca and 29% of Mg in the roots. Also, about 31% of total absorbed N, 17% of P, 14% of K, 43% of Ca and 36% of Mg was returned to the soil in fallen leaves.

Table 12. Total dry matter (g/plant) and nutrient contents (mg/plant) of fertilized and unfertilized MCol 22 [1)] during a 12-month growth cycle in Quilichao, Colombia, in 1982/83.

		Months after planting								
		1	2	3	4	5	6	8	10	12
DM	Fertilized	6	47	293	343	537	497	761	807	730
	Unfertilized	5	34	158	247	344	385	552	580	537
N	Fertilized	312	1833	5280	5077	6725	5269	8191	6191	7340
	Unfertilized	254	1308	2866	3349	3940	3662	6606	5127	6039
P	Fertilized	21	126	492	502	709	589	867	865	707
	Unfertilized	18	105	227	268	341	304	475	440	406
K	Fertilized	188	1229	3403	2603	3659	3087	4434	4322	3588
	Unfertilized	134	731	2334	1718	2261	2391	3048	2647	2600
Ca	Fertilized	67	452	1490	1647	2168	1164	2531	2200	2507
	Unfertilized	58	347	972	1083	1528	893	2081	1519	1578
Mg	Fertilized	28	178	518	537	538	518	919	814	891
	Unfertilized	28	143	302	370	370	370	828	612	718
S	Fertilized	28	133	339	295	260	175	843	171	477
	Unfertilized	23	102	248	259	307	169	828	225	474
B	Fertilized	0.14	0.94	5.72	2.70	2.29	2.40	5.45	3.74	6.20
	Unfertilized	0.09	0.65	1.43	1.65	1.41	1.73	3.09	2.00	3.61
Cu	Fertilized	0.10	0.87	2.02	2.34	3.81	2.26	3.78	3.43	3.45
	Unfertilized	0.09	0.73	1.37	1.86	2.71	1.67	3.11	2.51	3.49
Fe	Fertilized	6.3	58.9	88.5	479.8	73.2	62.7	188.1	165.7	169.0
	Unfertilized	7.7	58.2	65.0	89.8	59.3	54.4	167.1	107.8	124.1
Mn	Fertilized	2.1	19.1	56.6	64.0	110.8	42.2	84.4	60.1	70.5
	Unfertilized	2.0	11.6	32.2	34.6	63.0	28.5	48.3	34.6	49.1
Zn	Fertilized	0.5	5.4	8.8	12.4	11.9	9.0	15.2	15.8	16.5
	Unfertilized	0.4	4.9	8.1	7.5	9.7	9.5	12.3	11.8	15.6

[1)] excluding fallen leaves

Total DM produced in this experiment was less than half that produced in 1978/79. This also resulted in much less nutrient absorption and removal in the root harvest. Total nutrient absorption, including that in fallen leaves, was highest for N, followed by K and Ca, followed by Mg, while absorption of P was relatively low. Nutrients removed from the field in the root harvest was highest for N (71 kg/ha), followed by K (42 kg/ha), Ca (18 kg/ha), P (8 kg/ha) and Mg (6 kg/ha). Much of the absorbed N, Ca and Mg would be returned to the soil in the form of fallen leaves or plant tops.

Table 13. Distribution of dry matter (t/ha) and nutrients (kg/ha) between tops, roots and fallen leaves of fertilized MCol 22 during a 12-month growth cycle in Quilichao, Colombia, in 1982/83.

		Months after planting								
		1	2	3	4	5	6	8	10	12
DM	-tops	0.09	0.55	1.98	1.74	1.98	1.77	3.26	2.47	2.63
	-roots	0.00	0.18	2.59	3.62	6.41	6.00	8.63	10.14	8.77
	-fallen leaves	-	-	0.14	0.49	1.30	1.76	1.82	2.09	2.11
	Total	0.09	0.73	4.71	5.85	9.69	9.53	13.71	14.70	13.51
N	-tops	4.78	24.80	62.59	48.48	50.58	33.39	73.62	33.78	43.55
	-roots	0.09	3.84	19.91	30.84	54.50	48.94	54.36	62.95	71.14
	-fallen leaves	-	-	3.36	11.28	27.44	37.66	39.78	48.06	49.08
	Total	4.87	28.64	85.86	90.60	132.52	119.99	167.76	144.79	163.77
P	-tops	0.31	2.42	4.83	3.50	4.00	3.31	5.73	3.37	3.16
	-roots	0.02	0.48	2.86	4.34	7.08	6.03	7.81	10.14	7.89
	-fallen leaves	-	-	0.19	0.61	1.44	1.81	1.91	2.42	2.48
	Total	0.33	2.90	7.88	8.45	12.52	11.15	15.45	15.93	13.53
K	-tops	2.80	15.03	27.91	16.44	18.20	9.70	25.92	17.80	13.92
	-roots	0.14	4.17	25.27	24.23	38.97	38.53	43.36	49.73	42.14
	-fallen leaves	-	-	1.31	3.16	7.73	8.92	9.11	10.41	10.48
	Total	2.94	19.20	54.49	43.83	64.90	57.15	78.39	77.94	66.54
Ca	-tops	1.03	6.19	19.53	20.02	24.17	12.55	26.26	19.05	21.55
	-roots	0.02	0.87	3.75	5.72	9.70	5.64	13.28	15.33	17.62
	-fallen leaves	-	-	2.37	8.19	16.38	22.45	23.42	27.51	27.89
	Total	1.05	7.06	25.65	33.93	50.25	40.64	62.96	61.89	67.06
Mg	-tops	0.42	2.42	6.22	5.73	5.05	4.42	9.02	6.61	7.76
	-roots	0.02	0.36	1.87	2.66	3.36	3.67	5.34	6.11	6.16
	-fallen leaves	-	-	0.62	2.11	4.81	6.36	6.55	7.42	7.50
	Total	0.44	2.78	8.71	10.50	13.22	14.45	20.91	20.14	21.42

d. Changes in soil characteristics

Dry matter production and root yields of MCol 22 were much lower in 1982/83 than in 1978/79 on the same plot of land due to more adverse weather as well as deteriorating soil fertility conditions. There was only a minor change in pH, which varied from 3.9 to 4.2 during the crop cycle (**Table 14**). In spite of the application of 500 kg/ha of dolomitic lime the exchangeable Ca and Mg at the start of the trial in 1982 were lower than in 1979 (**Table 9**) and continued to decrease even more during the crop cycle, from 1.6 to about 0.7 meq Ca/100 g, and from 0.76 to 0.28 meq Mg/100 g. This was accompanied by an increase in exchangeable Al from about 3.5 to 4.2 meq Al/100 g, resulting in an increase in Al saturation from 60 to 80%. By the end of the second trial in 1983 the Ca and Mg levels were low but still above their critical levels, while the Al saturation was above the critical level. This contrasts with about 1.9 meq Ca, 0.98 meq Mg, and 3.35 meq Al/100 g, and an Al saturation of 52% at the end of the first trial in 1979 (**Table 9**).

Similarly, **Table 14** and **Figure 12** show that after the incorporation of NPK fertilizers the soil inorganic N, available P and exchangeable K levels were quite high in

fertilized plots during the first month after planting in March 1982, but declined rapidly during the course of the 12-month growth cycle in both fertilized and unfertilized plots. At time of root harvest at 12 MAP, soil inorganic N was actually slightly lower in the fertilized than the unfertilized plot, while the available P and exchangeable K levels were slightly higher in the fertilized plots. In the fertilized plots the soil P and K levels were both slightly below their critical levels, while in the unfertilized plots they were well below the critical levels. Thus, it is clear that in these highly P-fixing soils with low K-supplying capacity, rather high levels of fertilization and some lime are required to prevent soil fertility decline and to maintain high yields of cassava.

Table 14. Change in soil chemical characteristics during a 12-month growth cycle of fertilized and unfertilized plants of MCol 22 in Quilichao, Colombia, in 1982/83.

	Months after planting								
	1	2	3	4	5	6	8	10	12
pH									
-fertilized	3.92	4.02	3.92	3.87	4.02	4.12	3.97	4.12	4.27
-unfertilized	4.02	4.17	4.07	4.02	4.05	4.17	4.07	4.07	4.17
OM (%)									
-fertilized	8.39	8.77	9.58	8.47	8.64	8.01	8.07	6.55	6.42
-unfertilized	8.79	9.32	9.29	8.71	8.53	8.18	8.25	6.93	6.65
NO_3+NH_4-N (ppm)									
-fertilized	105.0	88.0	90.2	79.2	45.5	51.0	52.5	38.7	29.5
-unfertilized	56.0	57.0	65.0	57.7	50.2	52.0	46.0	42.0	35.5
Avail. P (ppm)									
-fertilized	33.1	27.8	36.5	32.1	25.8	25.6	15.2	5.7	4.0
-unfertilized	5.2	4.0	6.2	6.9	3.5	5.6	2.6	2.6	1.4
Exch. K (meq/100 g)									
-fertilized	0.41	0.35	0.34	0.31	0.26	0.22	0.17	0.12	0.13
-unfertilized	0.27	0.24	0.22	0.26	0.23	0.19	0.15	0.10	0.11
Exch. Ca (meq/100 g)									
-fertilized	1.67	1.61	1.93	1.87	1.72	1.55	1.43	0.82	0.71
-unfertilized	1.52	1.62	1.67	1.87	1.48	1.39	1.26	0.92	0.69
Exch. Mg (meq/100 g)									
-fertilized	0.69	0.60	0.75	0.67	0.59	0.62	0.48	0.24	0.30
-unfertilized	0.69	0.70	0.76	0.75	0.60	0.60	0.47	0.29	0.26
Exch. Al (meq/100 g)									
-fertilized	3.47	4.22	3.70	4.15	3.77	3.62	3.87	4.35	3.95
-unfertilized	3.65	3.97	3.60	4.00	3.82	3.87	3.97	4.47	4.30
Al-saturation (%)									
-fertilized	56	62	55	59	59	60	65	79	78
-unfertilized	60	61	58	58	62	64	68	77	80

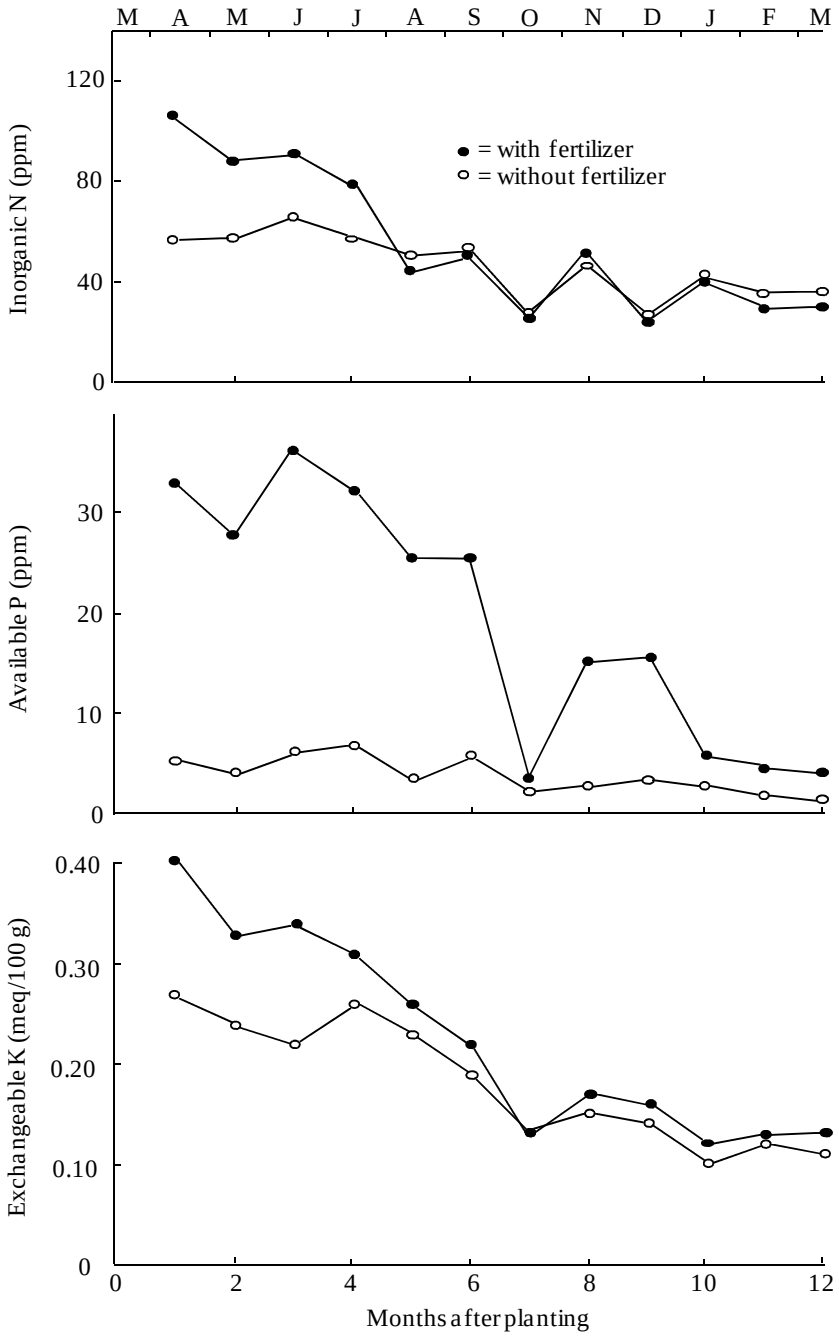

Figure 12. Change in inorganic N, available P and exchangeable K content of the soil during a 12-month growth cycle of fertilized and unfertilized cassava, MCol 22, in Quilichao, Colombia in 1982/83.

3. First experiment on nutrient absorption and distribution in Carimagua in 1983/84

Nutrient absorption by cassava during its 12-month growth cycle depends on soil fertility and moisture availability. These effects were studied in Carimagua (Agronomy field) where cassava, MVen 77, was grown with four treatments: with and without applied fertilizers (1 ton 10-20-20 and 5 kg Zn/ha) and with and without gravity irrigation during the four months of dry season, each with four replications. Subplots of six plants were harvested each month in such a way that the remaining subplots were always surrounded by border plants. The harvested plants were subdivided into 13 samples, consisting of leaf blades, petioles or stems of the upper, middle and lower part of the plant, as well as fibrous roots, tuberous roots and fallen leaf blades and petioles. The plant parts were weighed fresh, oven-dried and analyzed for major and minor elements. In the same subplots soil samples were taken to 20 cm depth and analyzed for available nutrients. Soil cores were also taken to one meter depth, and divided into 20 cm segments. Cassava roots in each segment were weighed and their mycorrhizal infection determined, while mycorrhizal spores were counted in the soil cores.

a. Dry matter production and distribution

Figure 13 shows the dry matter accumulation during the 12-month growth cycle as affected by fertilization and irrigation. Cassava was planted in early November, about a month before the onset of the dry season. While rainfall was low from December to April, it never ceased to rain completely, as normally occurs in Carimagua. Thus, plant growth was not markedly affected by lack of soil moisture, and supplemental irrigation had only a minor effect. Application of fertilizer increased plant growth more markedly, and at time of harvest had increased total dry matter (DM) 44% and root DM 47%. However, since the trial was located on a plot fertilized in previous years, the effect of fertilization was not as marked as would be expected in a virgin soil. DM accumulation was slow initially, but after the second month proceeded at a nearly constant rate until harvest.

Figure 14 shows that initially DM accumulated mainly in leaves and stems, but already after the third month the tuberous roots became the major sink, increasing steadily in weight until harvest. Due to excellent climatic conditions and good management fresh root yields in irrigated plots were as high as 43 t/ha with fertilization, and 28 t/ha without fertilization. Without irrigation, yields were 32 and 24 t/ha with and without fertilization, respectively. The effect of fertilization was significant, that of irrigation was not, partially due to the unusually wet dry season.

Table 15 shows the dry matter distribution among various plant parts of fertilized and unfertilized plants, both without irrigation, during the growth cycle. In this case, fertilizer application increased both total DM production and DM in roots about 30%. At time of harvest, of the total DM produced, 61-62% was found in the roots, about 25% in stems, 8-9% in the accumulated fallen leaves and only 4-5% in the leaf blades and petioles remaining on the plant. These proportions were about the same for fertilized and unfertilized plants.

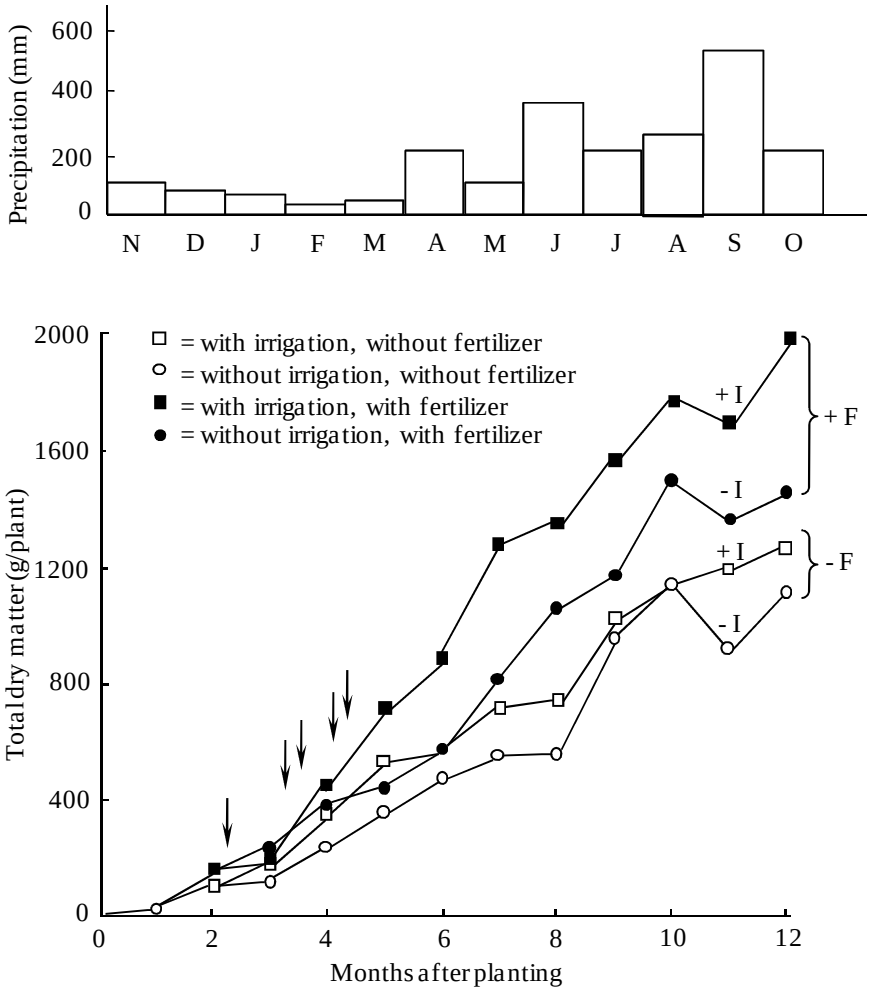

Figure 13. Monthly precipitation and accumulative total dry matter of cassava, MVen 77, as affected by fertilization and furrow irrigation during a 12-month growth cycle in Carimagua, in 1983/84. Arrows indicate when irrigation was applied.

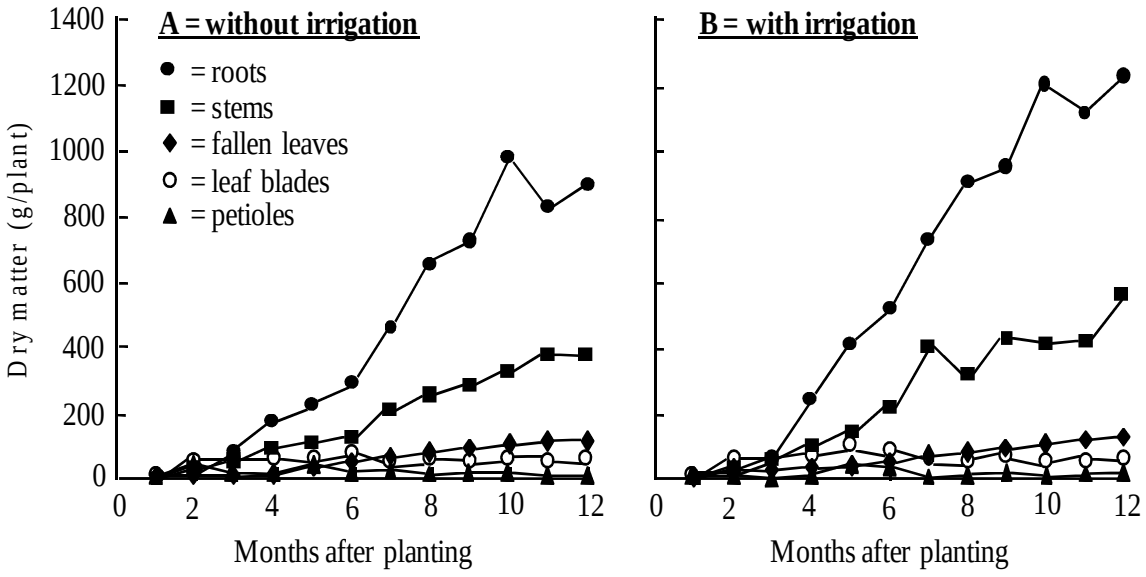

Figure 14. Dry matter distribution among roots, stems, leafblades, petioles and fallen leaves of fertilized cassava during a 12-month growth cycle in Carimagua, Colombia; with (B) or without (A) irrigation.
Source: *CIAT, 1985.*

Table 15. Dry matter production and distribution (g/plant) among various plant parts of fertilized and unfertilized cassava, MVen 77, both without irrigation, during a 12-month growth cycle in Carimagua, Colombia, in 1983/84.

	Months after planting								
	1	2	3	4	5	6	8	10	12
Unfertilized									
-leaf blades	5.5	36.0	40.8	44.1	36.4	39.4	37.8	40.5	36.7
-petioles	2.5	21.3	16.0	16.6	11.8	12.2	9.6	9.1	8.3
-stems	2.6	24.0	25.9	50.7	65.7	79.7	109.9	238.2	282.5
-roots	0.8	8.8	31.7	115.8	208.4	295.9	327.8	744.3	688.2
-fallen leaves[1)]	-	-	0.3	7.0	26.7	41.7	60.4	91.9	99.4
Total	11.5	90.2	114.6	234.2	349.1	468.8	545.6	1,124.0	1,115.1
Fertilized									
-leaf blades	5.4	60.2	61.2	61.1	56.8	69.9	59.7	60.9	58.5
-petioles	2.7	33.3	26.5	25.4	21.5	22.2	15.2	23.3	13.9
-stems	2.6	35.7	55.6	96.9	109.3	124.8	251.0	326.8	369.7
-roots	1.3	22.7	82.3	180.6	225.5	298.1	655.8	972.2	894.1
-fallen leaves[1)]	-	-	1.1	11.2	26.4	48.1	74.8	107.4	119.3
Total	12.0	151.9	226.7	375.2	439.4	563.2	1,056.6	1,490.6	1,455.5

[1)] accumulated fallen leaves

b. Nutrient concentrations in plant tissues

Table 16 shows the average nutrient concentrations in different plant parts at 3 and 4 MAP corresponding to the initial period of rapid growth of the plants. Similar to the results of the previous trials in Quilichao, the concentration of N, P, K and S were highest in the leaf blades, followed by stems, petioles and tuberous roots, and decreased from the upper to the lower part of the plants. In contrast, the Ca and Mg concentrations were highest in the petioles and stems followed by the leaf blades and tuberous roots; they increased from the upper to the lower part of the plant for the leaf blades and petioles, but the Ca concentration decreased in the stem while the Mg concentration was rather similar throughout the stem. Fallen leaf blades and petioles had the highest concentrations of Ca, but the lowest of N, P and K; this is due to the low mobility of Ca in the phloem as compared to that of N, P and K; the latter are considered mobile nutrients which are more readily mobilized from the old to the new leaves. The concentrations of all nutrients in upper leaf blades were at, or slightly above, the critical levels established in YFEL-blades at 3-4 MAP (Howeler, 2002), except that the Fe concentrations were either high or very high.

Table 16. Nutrient concentrations in various plant parts of fertilized and unfertilized cassava, MVen 77, in Carimagua, Colombia, in 1983/84. Data are average values for irrigated and non-irrigated plants at 3 and 4 MAP.

	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
	(%)						(ppm)				
Fertilized											
Leaf blades											
-upper	5.19	0.38	1.61	0.76	0.28	0.30	298	177	47	10.6	26
-middle	4.00	0.28	1.36	1.08	0.27	0.26	430	207	63	9.6	30
-lower	3.55	0.24	1.30	1.40	0.22	0.23	402	220	77	8.5	37
-fallen[1)]	1.11	0.14	0.54	1.88	0.23	0.19	3333	247	120	8.9	38
Petioles											
-upper	1.49	0.17	2.18	1.58	0.36	0.10	87	238	33	4.9	17
-middle	0.84	0.09	1.84	2.58	0.41	0.07	88	359	49	3.0	14
-lower	0.78	0.09	1.69	3.54	0.42	0.07	95	417	70	3.2	15
-fallen	0.69	0.06	0.82	3.74	0.20	0.08	294	471	155	3.1	17
Stems											
-upper	2.13	0.23	2.09	2.09	0.47	0.14	94	140	37	9.8	14
-middle	1.57	0.21	1.26	1.30	0.26	0.11	110	120	46	10.8	12
-lower	1.37	0.28	1.14	1.31	0.23	0.09	210	99	36	10.0	10
Roots											
-fibrous roots[1)]	1.71	0.19	1.03	0.71	0.33	0.20	3780	368	136	-	10
-tuberous roots	0.88	0.14	1.05	0.16	0.06	0.05	127	15	15	3.9	4
Unfertilized											
Leaf blades											
-upper	4.57	0.34	1.29	0.68	0.25	0.29	198	128	49	9.9	26
-middle	3.66	0.25	1.18	1.08	0.27	0.25	267	185	66	8.7	37
-lower	3.31	0.21	1.09	1.48	0.25	0.25	335	191	89	7.6	42
-fallen[1)]	2.31	0.13	0.50	1.69	0.25	0.22	4850	209	121	9.4	39
Petioles											
-upper	1.50	0.17	1.60	1.32	0.37	0.10	79	172	40	4.4	16
-middle	0.70	0.10	1.32	2.20	0.43	0.10	76	304	72	2.9	15
-lower	0.63	0.09	1.35	2.69	0.45	0.13	92	361	110	2.8	15
-fallen	0.54	0.05	0.54	3.52	0.41	0.13	271	429	94	2.5	18
Stems											
-upper	1.64	0.20	1.22	1.53	0.32	0.19	133	115	36	9.7	14
-middle	1.03	0.18	0.87	1.45	0.30	0.16	74	103	39	8.9	13
-lower	0.78	0.21	0.81	1.19	0.32	0.16	184	95	54	7.9	10
Roots											
-fibrous roots[1)]	1.52	0.15	1.02	0.77	0.38	0.16	5985	191	165	-	10
-tuberous roots	0.42	0.10	0.71	0.13	0.06	0.05	127	10	16	3.0	4

[1)] Fallen leaves and rootlets were probably contaminated with micronutrients from the soil.

Source: *Howeler, 1985.*

Figure 15 shows that irrigation had little effect on the concentration of N, P and K in the upper leaf blades. This suggests that nutrient uptake was little affected by soil moisture status. However, in both irrigated and non-irrigated plots, the concentration of all three macro-nutrients decreased markedly during the dry months from February to March and increased markedly at the onset of regular rains in April. After the new growth flush in May, nutrient concentrations remained fairly constant during the rest of the growth cycle. The marked decrease in nutrient absorption during the dry season, with or without irrigation, is probably due to stomatal closing and decreased transpiration as a result of low relative humidity. In fact, measurements of leaf water potential during the dry season showed little change between 10 am (-13.4 ± 1.7 bar) and 1 pm (-13.5 ± 2.3 bar); however, stomatal conductance decreased markedly. This suggests that the stomata closed due to changes in air humidity and not as a result of soil moisture deficit. Thus, transpiration and water consumption must have been reduced. At the end of the growth cycle lack of irrigation had decreased both top and root growth about equally to 86% in unfertilized and to 72% in fertilized plants, compared to those in irrigated plots.

c. Nutrient uptake and distribution

Table 17 shows the total DM and nutrient accumulation in plants, either with or without irrigation and with or without fertilization, during the growth cycle. Furrow irrigation was applied only five times during the 3rd to 5th month after planting (see **Figure 13**), which increased DM production and the total absorption of all nutrients except that of Ca in fertilized plants. However, fertilizer application increased both DM and nutrient accumulation more markedly than irrigation, even that of Ca, Mg and S, which were not applied in the fertilizers. Nutrient absorption was highest for N and K, followed by Ca, Mg, P and S. **Table 18** shows the distribution of these nutrients between tops, roots and fallen leaves for fertilized but non-irrigated plants, while **Figure 16** shows the course of nutrient accumulation in fertilized and unfertilized plants during the 12-month growth cycle. Fertilized plants had higher nutrient contents than unfertilized plants from the second month onward, especially N, P, K and Ca; this was less so in case of Mg and S. Towards the end of the growth cycle the plant tops were the dominant sink for N, Ca, Mg, S and all micro-nutrients, while the roots were the dominant sink for P and K. **Table 19** shows that at time of harvest at 12 MAP the fertilized plants had 102 kg K, 67 kg N and 17 kg of P/ha in the roots, while in unfertilized plants this was only about half, i.e. 55 kg K, 30 kg N and 7.5 kg P, even though the DM accumulation in roots of unfertilized plants was only about 23% lower than that of fertilized plants. Thus, nutrient removal in the root harvest was not proportional to yield, but increased relatively more with increases in yield due to the higher nutrient concentrations in the roots of well-fertilized and higher yielding plants.

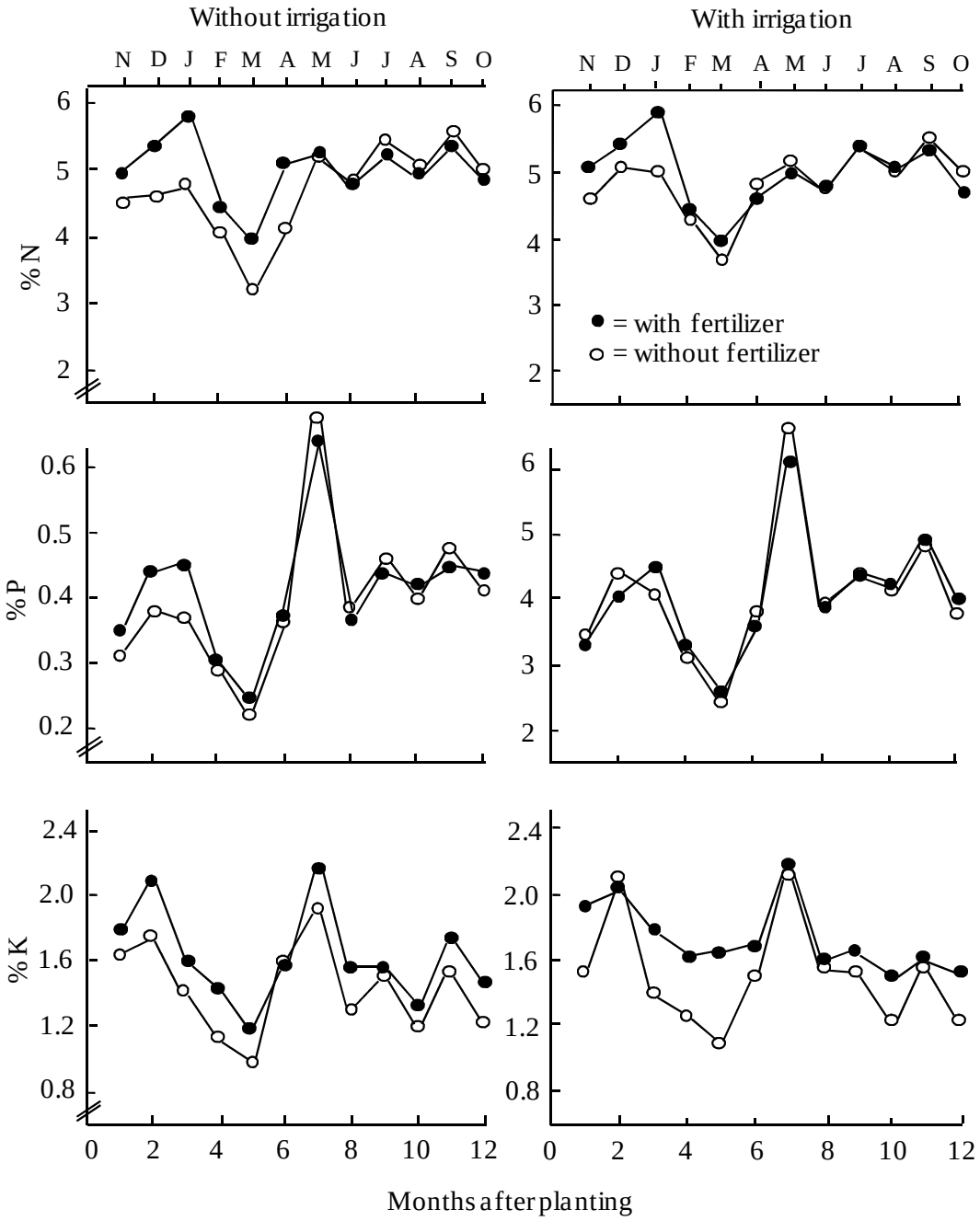

Figure 15. Change in concentration of N, P and K in upper leaf blades during a 12-month growth cycle of fertilized and unfertilized cassava, cv. MVen 77, grown in Carimagua with and without irrigation during the dry season in 1983/84.

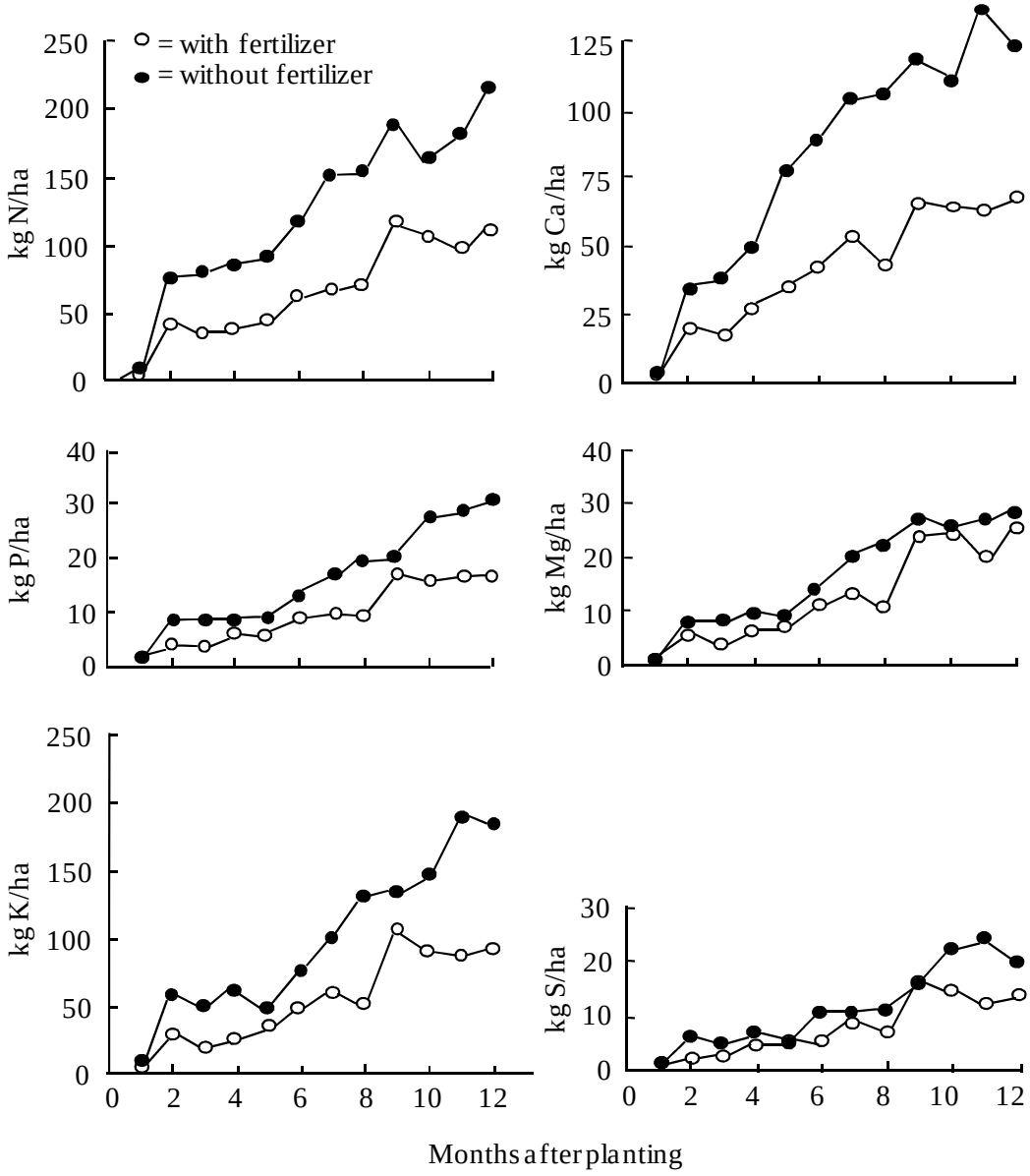

Figure 16. Accumulation of N, P, K, Ca, Mg and S in fertilized and unfertilized but non-irrigated cassava, cv. MVen 77, during a 12-month growth cycle in Carimagua, Colombia, in 1983/84.

d. Changes in soil characteristics

Figure 17 shows that the inorganic N, available P and exchangeable K contents of the soil decreased during the growth cycle. Fertilized plots had initially higher levels of soil nutrients than unfertilized plots, but these differences diminished with time due to increased nutrient absorption by fertilized plants, increased fixation of P and leaching of N and K. Fertilized plants accumulated 198 kg N, 30 kg P and 183 kg of K/ha, compared with 123 kg N, 16 kg P and 92 kg K/ha in unfertilized plants **(Table 19**). Of the total amounts of nutrients absorbed about 34% of N, 55% of P and 56% of K was removed in the root harvest of the fertilized plants, indicating again the large amounts of K removed with the harvest of cassava roots, resulting in a significant depletion of soil-K. **Table 20** shows the change in soil parameters during the cassava growth cycle. There was only a very slight increase in soil pH and exchangeable Ca, a slight decrease in Mg and a slight increase in exchangeable Al and Al-saturation. There was little change in total (organic + inorganic) N but only a slight build up during the dry season at the 4^{th} and 5^{th} month.

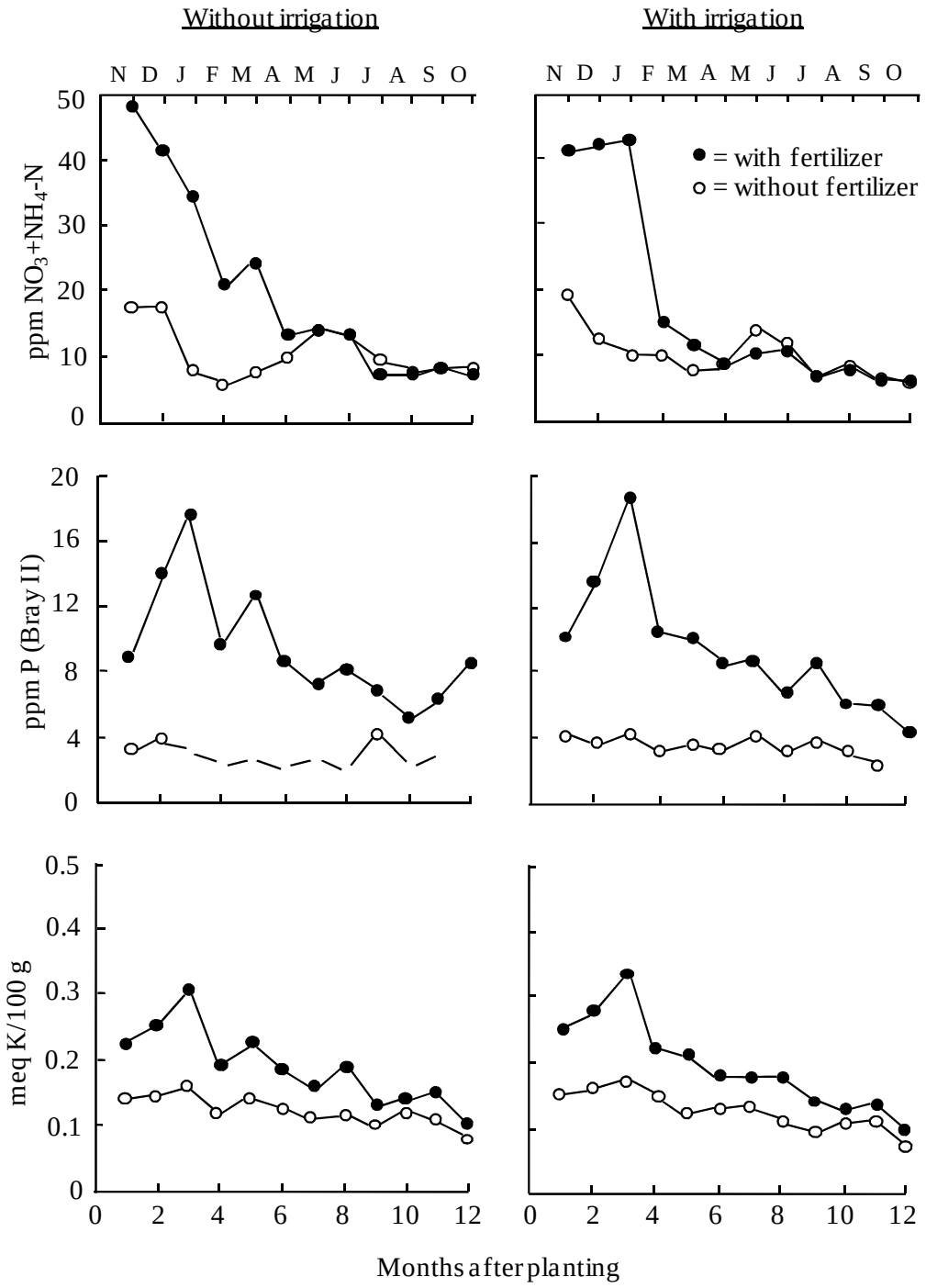

Figure 17. Change in nutrient concentrations of fertilized and unfertilized soil during a 12-month growth cycle of cassava, MVen 77, grown without and with irrigation in Carimagua, Colombia, in 1983/84.

Table 17. Effect of irrigation and fertilizer application on the total dry matter (DM) and nutrient accumulation (g/plant) during a 12-month growth cycle of cassava, MVen 77, in Carimagua, Colombia, in 1983/84.

	Months after planting								
	1	2	3	4	5	6	8	10	12
DM									
$I_0 F_0$ [1)]	11	90	115	234	349	469	546	1124	1115
$I_0 F_1$	12	152	227	375	439	563	1057	1491	1456
$I_1 F_0$	13	116	166	347	516	560	739	1121	1271
$I_1 F_1$	13	146	199	447	711	889	1359	1778	1986
N									
$I_0 F_0$	0.38	2.79	2.34	2.50	2.81	4.04	4.78	6.91	7.88
$I_0 F_1$	0.45	5.34	5.22	5.51	5.91	7.80	9.82	10.59	12.65
$I_1 F_0$	0.42	3.59	2.89	4.03	5.00	4.94	5.27	8.20	10.40
$I_1 F_1$	0.52	5.33	4.76	6.05	7.29	8.98	9.61	9.52	14.71
P									
$I_0 F_0$	0.03	0.27	0.24	0.32	0.37	0.59	0.61	1.01	1.05
$I_0 F_1$	0.03	0.57	0.56	0.56	0.55	0.85	1.24	1.75	1.95
$I_1 F_0$	0.03	0.41	0.31	0.46	0.48	0.92	0.97	1.22	1.20
$I_1 F_1$	0.03	0.54	0.54	0.65	0.88	1.10	1.68	2.13	2.53
K									
$I_0 F_0$	0.29	1.95	1.28	1.76	2.21	3.14	3.37	5.79	5.92
$I_0 F_1$	0.36	3.63	3.26	3.90	3.18	4.85	8.34	9.46	11.75
$I_1 F_0$	0.27	2.73	1.80	2.92	2.93	4.52	5.19	6.67	6.95
$I_1 F_1$	0.41	3.58	2.96	5.14	8.32	8.58	13.67	14.45	16.47
Ca									
$I_0 F_0$	0.17	1.31	1.11	1.77	2.26	2.79	2.72	4.08	4.32
$I_0 F_1$	0.19	2.27	2.43	3.25	4.98	5.71	6.75	7.55	8.35
$I_1 F_0$	0.19	1.71	1.46	2.19	2.59	3.26	3.28	4.37	4.42
$I_1 F_1$	0.22	2.43	2.02	3.20	4.59	5.29	6.53	6.29	8.24
Mg									
$I_0 F_0$	0.05	0.39	0.30	0.41	0.48	0.72	0.75	1.57	1.71
$I_0 F_1$	0.04	0.55	0.50	0.59	0.66	0.82	1.25	1.80	1.82
$I_1 F_0$	0.04	0.54	0.36	0.55	0.78	0.90	1.08	1.74	1.81
$I_1 F_1$	0.04	0.51	0.45	0.67	0.99	1.23	1.60	1.83	2.37
S									
$I_0 F_0$	0.02	0.17	0.18	0.34	0.31	0.44	0.48	0.92	0.89
$I_0 F_1$	0.02	0.49	0.29	0.44	0.34	0.61	0.67	1.35	1.23
$I_1 F_0$	0.02	0.26	0.18	0.46	0.45	0.52	0.76	1.29	1.24
$I_1 F_1$	0.03	0.24	0.23	0.48	0.68	0.74	1.12	1.57	1.79

[1)] I_0 = without irrigation; I_1 = with irrigation
F_0 = without fertilizer; F_1 = with fertilizer

Table 18. Nutrient distribution between tops, roots and fallen leaves (g or mg/plant) of fertilized but non-irrigated cassava, MVen 77, during a 12-month growth cycle in Carimagua, Colombia, in 1983/84.

	Months after planting								
I_oF_1	1	2	3	4	5	6	8	10	12
N (g/plant)									
-tops	0.45	5.0	4.4	4.0	3.9	5.3	5.4	6.1	6.4
-roots	0	0.34	0.7	1.3	1.6	1.9	3.2	2.7	4.3
-fallen leaves	-	-	0.04	0.18	0.34	0.66	1.13	1.70	1.95
Total	0.45	5.34	5.22	5.51	5.91	7.80	9.82	10.59	12.65
P (g/plant)									
-tops	0.03	0.53	0.44	0.33	0.28	0.42	0.51	0.67	0.75
-roots	0	0.04	0.11	0.22	0.25	0.39	0.66	0.97	1.07
-fallen leaves	-	-	0	0.01	0.02	0.04	0.07	0.11	0.13
Total	0.03	0.57	0.55	0.56	0.55	0.85	1.24	1.75	1.95
K (g/plant)									
-tops	0.33	3.28	2.35	2.23	1.63	2.61	3.78	3.71	4.76
-roots	0.03	0.35	0.89	1.61	1.45	2.09	4.28	5.35	6.54
-fallen leaves	-	-	0	0.05	0.10	0.15	0.27	0.38	0.46
Total	0.36	3.63	3.26	3.90	3.18	4.85	8.34	9.46	11.75
Ca (g/plant)									
-tops	0.18	2.19	2.31	2.67	2.29	2.53	2.92	3.27	3.52
-roots	0.01	0.08	0.11	0.26	0.21	0.34	0.60	0.59	0.99
-fallen leaves	-	-	0.01	0.31	2.47	2.84	3.24	3.16	3.29
Total	0.19	2.27	2.43	3.25	4.98	5.71	6.75	7.02	7.80
Mg (g/plant)									
-tops	0.03	0.51	0.44	0.46	0.46	0.52	0.70	0.93	0.98
-roots	0.01	0.04	0.06	0.09	0.12	0.15	0.33	0.59	0.54
-fallen leaves	-	-	0	0.03	0.08	0.14	0.21	0.28	0.30
Total	0.04	0.55	0.50	0.58	0.66	0.81	1.24	1.80	1.82
S (g/plant)									
-tops	0.02	0.44	0.25	0.29	0.21	0.31	0.36	0.62	0.61
-roots	0	0.04	0.03	0.13	0.09	0.24	0.20	0.59	0.45
-fallen leaves	-	-	0	0.02	0.03	0.06	0.10	0.15	0.17
Total	0.02	0.48	0.28	0.44	0.34	0.61	0.67	1.35	1.23
B (mg/plant)									
-tops	0.2	2.6	2.8	3.3	3.4	3.5	6.8	6.1	5.4
-roots	0	0.3	0.3	0.5	1.1	1.3	1.3	4.8	4.5
-fallen leaves	-	-	0	0.8	0.9	1.4	2.0	2.7	3.0
Total	0.2	2.9	3.2	4.7	5.4	6.3	10.2	13.7	12.8
Cu (mg/plant)									
-tops	0.1	1.7	1.5	1.3	1.1	1.5	1.8	2.4	2.2
-roots	0	0.6	0.3	0.8	0.7	0.9	1.2	4.0	1.9
-fallen leaves	-	-	0	0	0.1	0.3	0.5	0.8	0.9
Total	0.1	2.3	1.8	2.2	1.9	2.7	3.5	7.2	5.0
Fe (mg/plant)									
-tops	2.0	12.5	14.4	18.8	26.2	42.8	31.7	68.0	29.1
-roots	10.6	16.5	10.9	27.5	25.6	41.6	65.4	58.0	57.5
-fallen leaves	-	-	4.1	17.2	30.1	111.3	156.7	230.5	251.3
Total	12.6	36.7	54.5	75.3	101.3	277.5	345.7	374.1	358.9

Table 18. Continued

	Months after planting								
I_oF_1	1	2	3	4	5	6	8	10	12
Mn (mg/plant)									
-tops	1.3	23.2	26.7	31.2	24.7	27.9	30.9	36.2	36.8
-roots	0.6	1.9	1.2	2.6	1.2	2.1	3.6	2.3	3.8
-fallen leaves	-	-	0.4	3.9	7.9	13.7	21.1	27.2	29.8
Total	1.9	25.2	28.1	37.7	33.8	43.7	55.7	65.8	70.3
Zn (mg/plant)									
-tops	2.2	6.7	5.7	6.0	5.4	6.6	8.4	13.3	16.5
-roots	0.3	0.8	0.6	2.2	2.0	3.8	3.3	6.3	6.3
-fallen leaves	-	-	0	0.9	2.4	4.0	6.4	10.5	11.8
Total	2.5	7.5	6.3	9.0	9.8	14.5	18.1	30.2	34.6

[1)] the fallen leaves were probably contaminated with Fe from the soil.

Table 19. DM and nutrient distribution in 12-month old cassava, MVen 77, grown with and without fertilization and without irrigation in Carimagua, Colombia, in 1983/84.

	(t/ha)	(kg/ha)										
	DM	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
Unfertilized												
-Tops	5.11	69.1	7.4	33.6	37.4	16.2	8.2	0.07	0.03	0.45	0.33	0.26
-Roots	10.75	30.3	7.5	54.9	5.4	6.5	3.3	0.08	0.02	0.38	0.02	0.10
-Fallen leaves	1.55	23.7	1.5	4.0	24.7	4.0	2.5	0.04	0.01	-	0.37	0.18
Total	**17.41**	**123.1**	**16.4**	**92.5**	**67.5**	**26.7**	**14.0**	**0.19**	**0.06**	**-**	**0.72**	**0.54**
Fertilized												
-Tops	6.91	99.9	11.7	74.3	55.0	15.3	9.6	0.08	0.03	0.78	0.57	0.30
-Roots	13.97	67.3	16.8	102.1	15.5	8.4	7.0	0.07	0.03	0.90	0.06	0.17
-Fallen leaves	1.86	30.5	2.0	7.1	31.9	4.7	2.6	0.05	0.02	-	0.46	0.19
Total	**22.74**	**197.7**	**30.5**	**183.5**	**102.4**	**28.4**	**19.3**	**0.20**	**0.08**	**-**	**1.09**	**0.66**

Source*: *Howeler, 1985a.

e. Change in mycorrhizal infection

In the monthly sampling of soil with a 7 cm diameter drill down to one meter depth, very few fibrous roots of cassava were encountered beyond the top 20 cm depth, indicating the sparcity of the cassava root system and its presence principally in the fertilized topsoil. Apparently, only a few roots penetrated to deeper layers for water uptake during the dry season. The few roots recovered by this sampling technique showed a low level of mycorrhizal infection from 0 to 60%.

Table 20. Change in soil characteristics during a 12 months crop cycle of cassava, MVen77, grown with or without irrigation and with or without fertilizers in Carimagua, Colombia, in 1983/84.

	Months after planting								
	1	2	3	4	5	6	8	10	12
pH									
-I_0 F_0 [1)]	4.8	4.7	4.9	5.0	5.1	4.8	4.7	5.0	4.8
-I_0 F_1	4.5	4.4	4.4	4.6	4.6	4.6	4.7	4.9	4.8
-I_1 F_0	4.8	5.0	4.8	4.8	4.8	4.8	4.8	5.2	4.9
-I_1 F_1	4.6	4.5	4.4	4.8	4.7	4.8	4.9	5.1	5.0
NO_3+NH_4 (ppm)									
-I_0 F_0	17.5	17.5	8.0	5.7	7.5	9.7	13.4	8.2	8.0
-I_0 F_1	48.1	41.8	34.0	20.8	24.1	13.5	13.3	8.2	7.3
-I_1 F_0	19.4	12.6	10.4	10.4	8.2	8.1	12.5	9.0	5.5
-I_1 F_1	41.1	42.3	43.2	15.0	12.0	8.6	11.0	8.0	6.3
Total N (ppm)									
-I_0 F_0	1204	1064	1260	1372	1344	1288	1123	1204	1372
-I_0 F_1	1176	1176	1260	1428	1316	1316	1204	1204	1316
-I_1 F_0	1176	1192	1204	1316	1316	1232	1120	1148	1232
-I_1 F_1	1204	1148	1204	1232	1204	1260	1064	1120	1176
P (ppm)									
-I_0 F_0	3.3	3.8	3.3	2.4	2.7	2.4	2.0	2.2	2.5
-I_0 F_1	8.9	14.0	17.6	9.7	12.8	8.6	8.4	5.2	8.6
-I_1 F_0	4.3	3.8	4.3	3.3	3.6	3.4	3.1	3.1	4.0
-I_1 F_1	10.2	13.6	18.8	10.6	10.3	8.8	6.8	6.1	4.3
K (meq/100 g)									
-I_0 F_0	0.14	0.14	0.16	0.12	0.14	0.13	0.12	0.12	0.08
-I_0 F_1	0.22	0.25	0.31	0.19	0.23	0.19	0.19	0.14	0.10
-I_1 F_0	0.15	0.16	0.17	0.15	0.12	0.13	0.11	0.11	0.07
-I_1 F_1	0.25	0.28	0.34	0.22	0.21	0.18	0.18	0.13	0.09
Ca (meq/100 g)									
-I_0 F_0	0.76	0.86	1.11	0.99	1.07	1.29	0.94	0.90	0.87
-I_0 F_1	0.89	1.06	1.53	1.35	1.75	1.56	1.12	0.98	1.09
-I_1 F_0	1.14	1.11	1.10	0.94	0.86	1.07	1.11	1.28	0.91
-I_1 F_1	1.24	1.36	1.39	1.22	1.04	1.37	1.16	1.07	1.21
Mg (meq/100 g)									
-I_0 F_0	0.31	0.30	0.43	0.33	0.39	0.39	0.34	0.31	0.27
-I_0 F_1	0.35	0.36	0.43	0.33	0.39	0.32	0.33	0.29	0.32
-I_1 F_0	0.45	0.35	0.39	0.35	0.32	0.36	0.32	0.40	0.29
-I_1 F_1	0.41	0.42	0.43	0.39	0.32	0.38	0.31	0.31	0.35
Al (meq/100 g)									
-I_0 F_0	2.4	2.5	2.0	2.2	2.0	2.2	2.3	2.5	2.5
-I_0 F_1	2.4	2.4	2.0	2.1	2.0	2.2	2.3	2.5	2.3
-I_1 F_0	1.9	2.0	2.1	2.3	2.4	2.5	2.1	1.8	2.3
-I_1 F_1	1.8	1.8	1.7	1.9	2.1	2.1	1.9	2.2	2.0
Al-saturation (%)									
-I_0 F_0	66	65	53	60	56	55	61	64	66
-I_0 F_1	63	59	47	53	43	52	58	64	60
-I_1 F_0	53	55	55	61	64	61	58	50	64
-I_1 F_1	49	46	45	51	57	51	54	59	55

[1)] I_0 = without irrigation; I_1 = with irrigation F_0 = without fertilizer; F_1 = with fertilizer

Table 21 indicates that mycorrhizal spores were mainly present in the topsoil at the early stages of growth, but increased with soil depth during the dry season due to lack of soil moisture and/or cassava roots in the topsoil. After the onset of the rainy season in May, spore numbers increased again in the top layers of soil. Toward the end of the growth cycle maximum numbers of spores were found at the 60-80 cm depth. Since mycorrhizal spores only multiply rapidly in the vicinity of living roots, their presence indicates some root activity at a deeper depth, although the sampling technique did not actually encounter roots in any of the 16 cores taken monthly during the last half of the growth cycle.

Table 21. Mycorrhizal spores at different soil depths during a 12-month growth cycle of Cassava, MVen 77, in Carimagua, Colombia, in 1983/84.

Soil depth	Number of spores/100 g dry soil											
(cm)	D	J	F	M	A	M	J	J	A	S	O	N
0-20	-	120	27	2	20	35	147	200	197	676	388	244
20-40	-	32	40	10	100	38	139	209	499	603	279	348
40-60	-	24	114	46	166	76	71	180	456	285	503	467
60-80	-	0	9	12	231	66	10	112	276	374	569	249
80-100	-	0	0	0	17	36	5	46	78	269	295	134

4. Second experiment on nutrient absorption and distribution in Carimagua in 1984/85

To determine the effect of fertilization and the effect of relative humidity during the dry season on cassava growth and nutrient absorption, another cassava experiment was established in a virgin soil in Yopare field of Carimagua in October 1984, towards the end of the rainy season. The experiment had four main plots of 169 plants, either with (W_1) or without (W_0) a surrounding windbreak of tall-growing elephant grass, and either with (F_1) or without (F_0) fertilization. In the four replicates of each treatment subplots of six plants were harvested each month in such a way that the remaining subplots were always surrounded by border plants. The wind break of elephant grass was intended to increase the relative humidity around the plants and thus keep the stomata open for a longer period of time during the dry season, which might increase yields. The fertilized plots received 1 t/ha of dolomitic lime, 1 t/ha of 10-20-20 fertilizers, 10 kg Zn/ha as ZnO, 1 kg B/ha as Borax and 10 kg S/ha as elemental sulfur, all broadcast and incorporated before planting. Each month the six harvested plants were divided into seven different parts, i.e. leaf blades of youngest fully expanded leaves, all remaining leaf blades, all petioles, stems, fibrous and tuberous roots, and fallen leaves. These were weighed fresh, carefully washed, oven dried, weighed again and analyzed for all macro- and micro-nutrients. In addition, two sets of soil samples were taken every month, one set about 30 cm from the plants within cassava rows and another set in the first (empty) subplots that had already been harvested in each treatment to measure changes in soil fertility in the absence of cassava plants.

In the 1984/85 crop year the dry season was unusually long and dry, while rainfall during the wet season was unusually high, with a maximum of 463 mm during May.

During the long dry season plants defoliated completely, while full foliage was quickly re-established with the first rains in April. These drastic changes between extreme dry and extreme wet had a profound effect on plant growth, nutrient concentrations in the plant and in the soil, as well as the mycorrhizal populations.

a. Dry matter production and distribution

Table 22 shows the effect of the four treatments on dry matter production and distribution among various plant parts during the growth cycle.

Table 22. Dry matter production and distribution (g/plant) among various plant parts during an 11-month growth cycle of cassava, MVen 77, grown with or without a windbreak of elephant grass[1)] and with or without fertilization in Carimagua, Colombia, in 1984/85.

	Months after planting								
Treatments	1	2	3	4	5	6	8	10	12
W_0F_0 [2)]									
-leaf blades	5.27	11.05	2.12	4.54	9.64	12.62	76.65	37.73	16.65
-petioles	1.64	3.69	0.56	0.97	2.81	4.19	20.89	9.43	3.82
-stems	1.58	4.15	2.14	6.88	17.88	14.79	61.74	116.76	145.00
-roots	0.35	3.09	6.58	26.06	45.36	41.30	77.88	384.44	325.11
-fallen leaves[3)]	-	-	0.20	0.81	9.73	9.73	9.73	68.38	96.25
Total	8.84	21.98	11.60	39.26	85.42	82.63	246.89	616.74	586.83
W_0F_1									
-leaf blades	12.75	20.54	2.97	4.26	10.56	24.00	125.74	60.48	25.19
-petioles	3.98	10.00	0.52	1.12	2.59	7.00	49.18	16.86	5.22
-stems	4.51	17.00	4.26	18.70	53.90	39.33	141.71	329.17	307.70
-roots	0.55	17.58	10.04	42.15	43.56	61.50	156.58	410.32	531.79
-fallen leaves[3)]	-	-	0.47	4.55	11.19	11.19	11.19	77.77	98.17
Total	21.79	65.12	18.26	70.78	121.80	143.02	484.40	894.60	968.07
W_1F_0									
-leaf blades	3.95	9.85	2.23	3.78	6.17	12.76	52.19	33.08	19.65
-petioles	1.30	3.28	0.49	0.87	1.82	3.61	16.88	9.14	4.62
-stems	1.51	4.91	0.88	7.77	19.33	12.67	53.20	145.66	129.10
-roots	0.29	4.87	3.92	30.49	40.34	40.27	79.21	348.97	281.77
-fallen leaves[3)]	-	-	0.13	3.55	12.91	12.91	12.91	62.14	85.83
Total	7.05	22.91	7.65	46.46	80.57	82.22	214.39	598.99	520.97
W_1F_1									
-leaf blades	13.48	26.75	2.67	4.11	11.13	27.72	148.50	67.26	26.47
-petioles	4.90	11.86	0.55	0.99	2.63	9.70	89.20	19.50	5.55
-stems	5.02	17.45	4.19	19.80	42.50	43.44	166.60	363.70	387.90
-roots	0.47	22.23	12.09	37.26	53.04	62.58	144.11	446.12	585.32
-fallen leaves[3)]	-	-	0.49	5.46	22.42	22.42	22.42	118.24	159.04
Total	23.87	78.29	19.99	67.62	131.72	165.86	570.83	1014.82	1164.28

[1)] a row of tall growing elephant grass surrounded the plots as a wind break

[2)] W_0 = without wind break; W_1 = with wind break
F_0 = without fertilizers; F_1 = with fertilizers

[3)] accumulative fallen leaves

Initially most DM was used for production of leaves and stems, but starting in the third month roots became the dominant sink. Due to the long and severe dry season (from Nov 15 to April 15 it rained only 86 mm) total DM production was very low during the first six months of the growth cycle, but increased rapidly with the onset of rains in April and May. Since the elephant grass windbreaks seemed to have very little effect on total DM production and distribution **Figure 18** shows only the effect of fertilizer application in the presence of the windbreaks. Fertilizer application nearly doubled total DM production as well as the DM in roots. This resulted in a fresh root yield of 22.85 t/ha with fertilizers *versus* only 11.00 t/ha without fertilizers; without windbreak these yields were 20.83 and 12.69 t/ha, respectively.

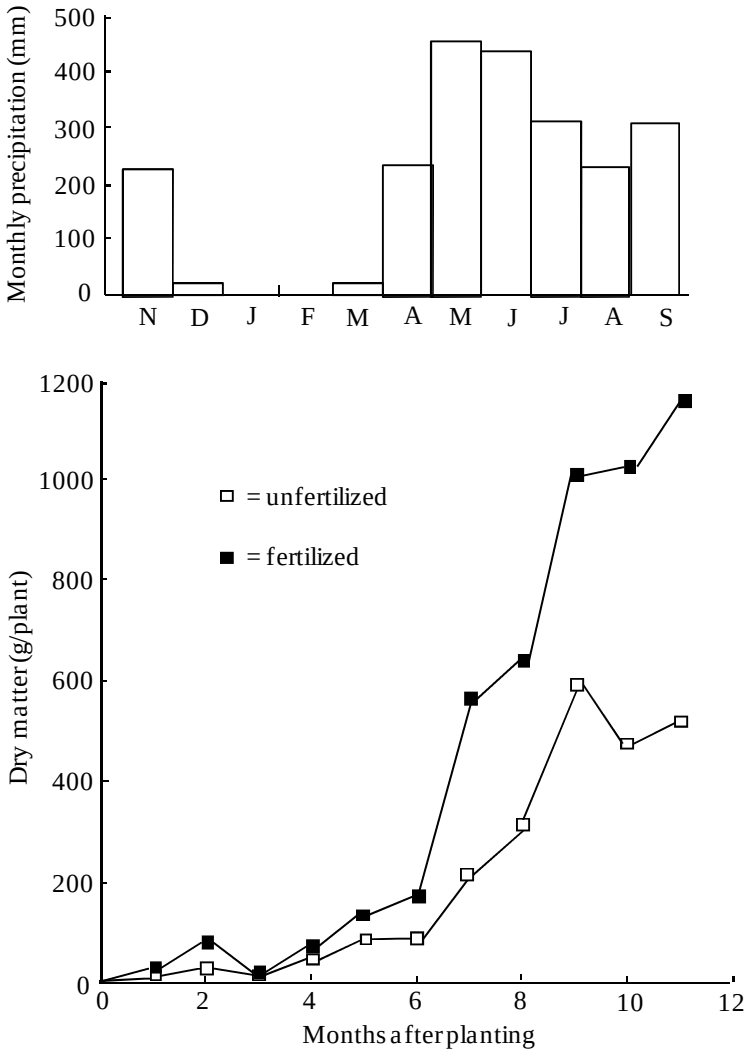

Figure 18. Monthly precipitation and accumulative total dry matter production in fertilized and unfertilized cassava, grown with an elephant grass windbreak during an 11-month growth cycle in Carimagua, Colombia, in 1984/85.

Figure 19 shows the dynamics of dry matter distribution between roots, stems, leaves and fallen leaves for fertilized plants. Initially most DM was used for production of leaves and stems, but starting in the third MAP the roots became the dominant sink. DM in leaves remained very low during the first six months due to slow new leaf production as a result of the severe drought. The drought also resulted in almost complete defoliation during the third MAP. At the onset of rains in April there was a very rapid regrowth of new leaves reaching a maximum of 238 g/plant in one month; this was followed by rapid growth of roots and stems with a simultaneous decline in leaf production and increase in leaf fall.

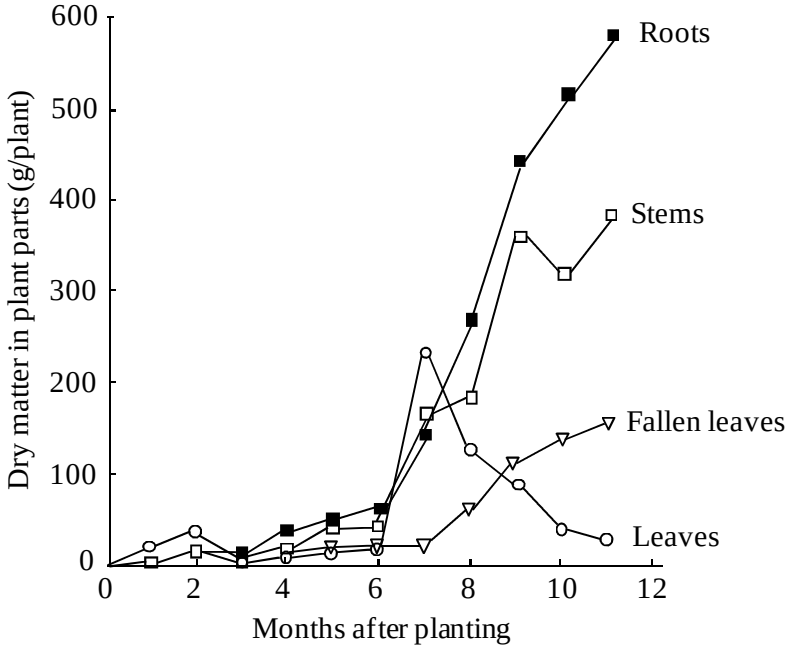

Figure 19. Dry matter distribution between different plant parts of cassava, MVen 77, during an 11-month growth cycle in fertilized soil and with windbreaks in Carimagua, Colombia, in 1984/85.

b. Nutrient concentration in plant tissues

Figure 20 shows the change in nutrient concentration in the youngest fully expanded leaf blades. Concentrations of N, P and K decreased during the dry season and increased markedly at the onset of rains, reaching a maximum at the sixth month. During the seventh month nutrient absorption rates still increased but the sudden increase in growth, especially leaves, resulted in a decrease in nutrient concentrations in the young leaves. It is clear that due to these drastic changes in nutrient concentrations, for diagnostic purposes these youngest leaves should not be sampled during the dry season, nor during the first two months of rain, but rather at re-initiation of leaf fall, at about the eighth month, or three months after the start of the rainy season. Even at this time, analysis of these youngest leaves in this trial did not give a good indication of nutrient deficiencies as there were relatively little differences in nutrient concentrations between the fertilized and unfertilized plants, even though the total DM production (**Figure 18**) and fresh root yields were markedly different. **Table 23** shows the average nutrient concentration of all different plant parts at 3 and 4 MAP. Concentrations of N and P were highest in young leaves and in the blades of mature leaves, followed by stems. In contrast, concentrations of K, Ca and Mg were highest in petioles and were relatively low in leaf blades and young leaves. The concentrations of N, P, K, Ca, Mg and S were all slightly below the critical levels determined for these nutrients in youngest fully expanded leaf (YFEL) blades (Howeler, 2002). The concentration of B was far below, and those of Fe, Mn and Zn were far above the critical levels of deficiency, while Fe concentrations were far above the critical level for toxicity established in nutrient solution. The concentrations of most nutrients were also considerably below those observed in the 1983/84 experiment (**Table 16**) due to the less severe dry season and better soil fertility conditions in that experiment.

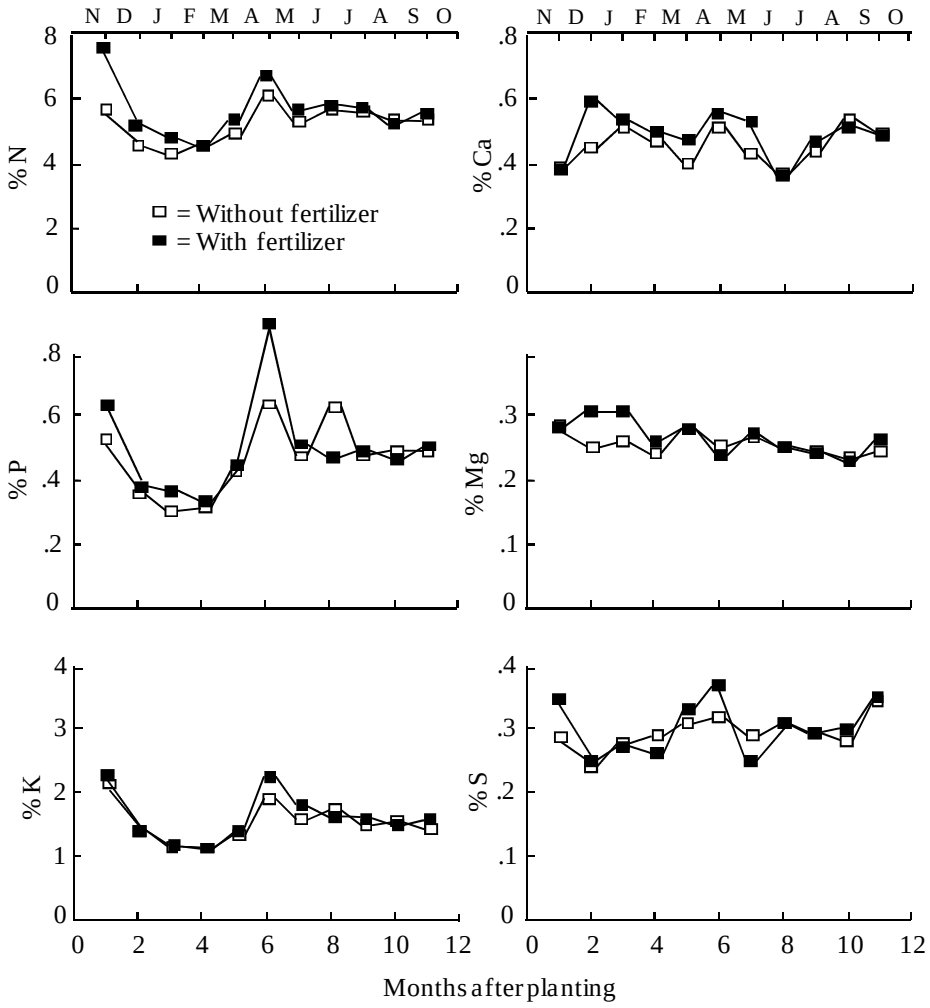

Figure 20. Change in nutrient concentrations in youngest fully expanded leaf blades of cassava, MVen 77, during an 11-month growth cycle in fertilized and unfertilized soil in Carimagua, in 1984/85 Data are the average of plots with and without elephant grass windbreaks.

Table 23. Nutrient concentration in various plant parts of fertilized and unfertilized cassava, MVen 77, in Carimagua in 1984/85. Data are average values for cassava grown with and without elephant grass windbreaks at 3 and 4 months after planting.

	(%)						(ppm)				
	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
Unfertilized											
-young leaves	4.47	0.30	1.08	0.50	0.25	0.29	608	106	70	8.6	16
-leaf blades	3.28	0.20	0.94	0.76	0.22	0.25	635	107	111	7.3	15
-petioles	1.03	0.11	1.53	1.56	0.36	0.12	191	205	153	4.0	13
-stems	1.76	0.17	0.95	1.60	0.33	0.16	156	90	130	10.4	8
-fibrous roots [1)]	1.12	0.11	1.59	0.48	0.23	0.17	2191	116	130	87.9	17
-tuberous roots	0.52	0.09	0.67	0.09	0.05	0.06	377	6	20	4.4	3
-fallen leaves	1.16	0.08	0.66	1.64	0.28	0.14	992	196	272	6.5	15
Fertilized											
-young leaves	4.74	0.34	1.06	0.52	0.28	0.26	649	127	73	9.4	16
-leaf blades	3.46	0.22	0.92	0.76	0.28	0.20	820	121	106	6.8	18
-petioles	1.22	0.11	1.74	1.58	0.50	0.08	197	221	99	4.2	14
-stems	2.62	0.26	1.31	1.13	0.18	0.10	128	62	88	9.7	9
-fibrous roots [1)]	1.79	0.15	2.15	0.52	0.30	0.12	3347	114	196	52.3	16
-tuberous roots	0.87	0.14	0.90	0.13	0.06	0.06	226	12	23	4.3	4
-fallen leaves	0.78	0.07	1.14	2.05	0.41	0.09	320	195	204	4.6	14

[1)] the fibrous roots were probably contaminated with soil

c. Nutrient uptake and distribution

Table 24 shows the total dry matter and nutrient accumulation for the four treatments during the 11-month growth cycle. Both dry matter and nutrient accumulations were about twice as high in the fertilized as in the unfertilized plants, but they were considerably lower than in the 1983/84 experiment. Among the nutrients, absorption of N was highest, followed by K, while that of P, Mg and S was much lower. **Table 25** shows the nutrient distribution at time of harvest for both the fertilized and unfertilized plants. At harvest about 54% of total absorbed K, 47% of P and only 23% of N were found in the tuberous roots. This means that about 66 kg of K, 11 kg of P and 33 kg of N were removed per ha in the root harvest of fertilized plants; for the unfertilized plants this was 25 kg of K, 14 kg of N and only 4 kg of P. Most of these nutrients were absorbed after the onset of the rainy season in April and May.

d. Changes in soil characteristics

Figure 21 and **Table 26** show the change in soil nutrient concentrations during the 11-month crop cycle. Available P concentrations nearly doubled during the dry season, both in fertilized and unfertilized plots. Fertilization with 1 t/ha of 10-20-20 resulted in an available P content of 38 ppm compared with 8 ppm without fertilization. Similarly, the exchangeable K contents increased during the dry season, while inorganic N initially decreased and then increased markedly towards the end of the dry season due to a build up of both NO_3- and NH_4-N. At the onset of rains at the end of April, N, P and K concentrations in the soil decreased rapidly due to leaching and increased plant growth and nutrient absorption.

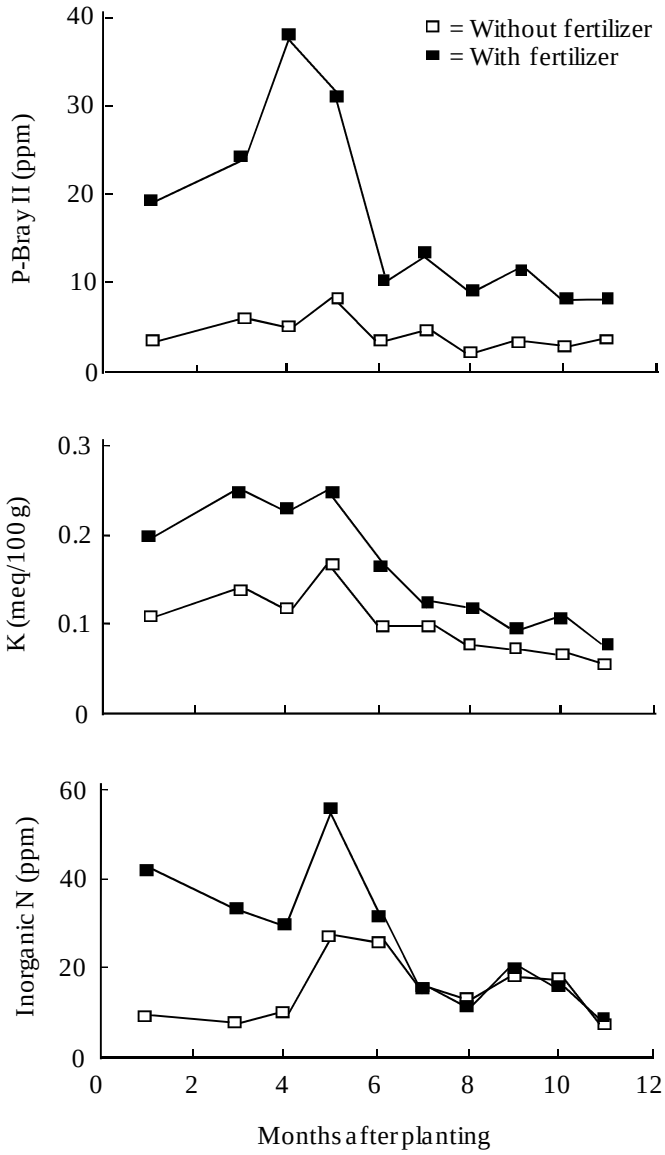

Figure 21. Change in nutrient concentrations of fertilized and unfertilized soil during an 11-month growth cycle of cassava, MVen 77, in Carimagua, in 1984/85. Data are the average of plots with and without elephant grass windbreaks.

Table 24. Effect of elephant grass wind breaks and fertilization on total dry matter (DM) and nutrient accumulation (g/plant) during an 11-month growth cycle of cassava, MVen 77, in Carimagua, Colombia, in 1984/85.

		Months after planting								
		1	2	3	4	5	6	7	9	11
DM	W_0F_0	8.8	22.0	11.6	39.3	85.4	82.6	246.9	616.7	587.8
	W_0F_1	21.8	65.1	18.3	70.8	121.8	143.0	484.4	894.6	968.1
	W_1F_0	7.0	22.9	7.6	46.5	80.6	82.2	214.4	599.0	521.0
	W_1F_1	23.9	78.3	20.0	67.6	131.7	165.9	570.8	1014.8	1164.3
N	W_0F_0	0.33	0.62	0.16	0.43	0.98	1.20	5.00	6.61	5.06
	W_0F_1	1.09	1.60	0.30	0.80	1.65	2.53	8.49	8.34	7.18
	W_1F_0	0.25	0.55	0.12	0.44	0.83	1.18	3.17	4.90	4.20
	W_1F_1	1.20	1.82	0.36	1.01	2.29	3.55	11.55	8.95	8.93
P	W_0F_0	0.03	0.05	0.02	0.04	0.09	0.10	0.34	0.55	0.49
	W_0F_1	0.08	0.14	0.03	0.01	0.17	0.30	0.80	0.94	1.13
	W_1F_0	0.02	0.05	0.01	0.05	0.08	0.06	0.26	0.56	0.54
	W_1F_1	0.10	0.15	0.04	0.12	0.18	0.35	1.01	1.13	1.50
K	W_0F_0	0.22	0.35	0.10	0.29	0.68	0.62	1.43	4.38	2.91
	W_0F_1	0.62	0.92	0.19	0.62	0.92	1.36	4.95	7.64	5.61
	W_1F_0	0.17	0.33	0.07	0.32	0.53	0.56	1.93	3.95	3.06
	W_1F_1	0.74	1.05	0.21	0.82	0.93	1.57	7.60	7.73	7.82
Ca	W_0F_0	0.09	0.22	0.13	0.20	0.48	0.51	1.50	2.02	2.19
	W_0F_1	0.26	0.66	0.11	0.40	0.70	0.94	2.86	4.25	3.04
	W_1F_0	0.07	0.21	0.04	0.23	0.41	0.46	1.10	2.10	1.84
	W_1F_1	0.27	0.73	0.11	0.42	0.92	1.29	3.93	4.66	4.01
Mg	W_0F_0	0.03	0.06	0.02	0.05	0.12	0.14	0.44	0.60	0.69
	W_0F_1	0.07	0.17	0.03	0.09	0.16	0.22	0.84	1.06	1.06
	W_1F_0	0.02	0.05	0.02	0.06	0.11	0.13	0.32	0.67	0.56
	W_1F_1	0.08	0.20	0.03	0.09	0.23	0.31	0.95	1.21	1.25
S	W_0F_0	0.02	0.04	0.01	0.04	0.08	0.09	0.32	0.48	0.59
	W_0F_1	0.05	0.08	0.02	0.05	0.11	0.15	0.51	0.73	0.95
	W_1F_0	0.01	0.04	0.01	0.05	0.08	0.09	0.26	0.59	0.63
	W_1F_1	0.06	0.09	0.02	0.07	0.14	0.24	0.64	1.02	1.22

W_o = without wind breaks; W_1 = with wind breaks
F_o = without fertilization; F_1 = with fertilization

After the flush of top and root growth, nutrient absorption leveled off or decreased (**Table 24).** Nutrient levels in the soil more or less stabilized around the seventh to eighth month. Fertilized plots had consistently higher levels of P and K, while the effect on inorganic N had nearly disappeared after the sixth month. In unfertilized plots there was essentially no net change in available P, there was a slight build up of inorganic N and a considerable decline in exchangeable K during the course of one crop cycle. Only with the application of 167 kg K/ha did K contents in the soil return to their original level of 0.11 meq/100 g. At the time of harvest there was a total K absorption of 122 kg/ha, of which 66 kg were removed in the root harvest.

Table 25. Nutrient distribution between top, roots and fallen leaves (mg/plant) of fertilized and unfertilized cassava[1)], MVen 77, at time of harvest at 11 MAP in Carimagua, Colombia, in 1984/85. Numbers in parentheses indicate the percent of each nutrient removed in the root harvest.

	Without fertilizers	With fertilizers
N -tops	2,201	4,903
-roots	872 (21%)	2,111 (24%)
-fallen leaves	1,126	1,917
Total	4,199	8,931
P -tops	211	660
-roots	253 (47%)	702 (47%)
-fallen leaves	78	139
Total	542	1,501
K -tops	1,233	3,078
-roots	1,607 (53%)	4,214 (54%)
-fallen leaves	220	523
Total	3,060	7,815
Ca -tops	759	1,443
-roots	141 (8%)	469 (12%)
-fallen leaves	935	2,100
Total	1,835	4,012
Mg -tops	247	624
-roots	169 (30%)	292 (23%)
-fallen leaves	143	331
Total	559	1,247
S -tops	307	600
-roots	197 (31%)	410 (34%)
-fallen leaves	127	209
Total	631	1,219

[1)] Data are from plots with wind breaks

In the absence of cassava the soil nutrient contents in fertilized plots tended to be slightly higher, while those in unfertilized plots tended to be lower, but both followed the same trends as in the corresponding plots with cassava. This indicates that the effect of the extreme dry season as well as the very wet rainy season seems to have had a more significant effect on soil fertility conditions than the nutrient uptake by the growth of cassava. An alternative explanation is that plants surrounding the empty subplots from which soil sample were collected had put their roots into these empty plots and were able to take up nutrients from these plots. While fertilizer application initially markedly increased the available N, P and K concentrations in the soil, at the onset of the rainy season these concentrations decreased more rapidly than in the unfertilized plots. By the end of the growth cycle the concentrations of P and K in fertilized plots were only slightly higher than in the unfertilized plots, and those of inorganic-N were essentially the same. **Table 26** also shows that the application of 1 t/ha of dolomiic lime in the fertilized plots more or less

doubled the concentrations of exchangeable Ca and Mg, which in turn decreased the exchangeable Al and the percent Al-saturation, but this had no consistent effect on soil pH. Both soil Ca and Mg concentrations increased during the dry season, but decreased again during the following wet season.

Table 26. Change in soil characteristics during an 11-month crop cycle of cassava [1], MVen 77, grown with or without fertilization in Carimagua, Colombia in 1984/85, as well as in the same plots without cassava [2].

		Months after planting								
		1	3	4	5	6	7	8	9	11
pH										
-with cassava	-F_0	4.82	4.76	4.92	5.10	4.81	4.57	4.57	4.47	4.66
	-F_1	4.44	4.48	4.78	4.95	4.85	4.69	4.73	4.63	4.66
-without cassava	-F_0	-	4.82	4.81	5.09	4.77	4.56	4.39	4.47	4.58
	-F_1	--	4.60	4.62	4.97	4.93	4.77	4.64	4.59	4.67
NO_3-N+NH_4-N (ppm)										
-with cassava	-F_0	9.2	8.7	10.1	27.0	25.7	16.3	13.9	18.2	8.7
	-F_1	41.8	33.5	29.4	55.4	31.1	15.5	12.0	20.2	9.2
-without cassava	-F_0	-	9.8	13.8	22.9	25.6	15.8	14.3	19.2	8.5
	-F_1	-	42.0	43.3	60.6	36.6	16.0	15.3	16.8	8.7
Avail. P (ppm)										
-with cassava	-F_0	3.2	6.2	5.3	8.1	3.4	4.5	2.4	3.5	3.7
	-F_1	19.1	24.5	37.9	31.0	10.1	13.4	9.6	11.5	8.3
-without cassava	-F_0	-	6.4	7.4	6.7	5.1	3.0	3.6	3.8	5.3
	-F_1	-	36.7	36.7	44.8	17.8	13.9	11.5	14.8	5.8
Exch. K (meq/100 g)										
-with cassava	-F_0	0.11	0.14	0.12	0.17	0.10	0.10	0.08	0.08	0.06
	-F_1	0.20	0.25	0.23	0.25	0.17	0.13	0.12	0.10	0.08
-without cassava	-F_0	-	0.14	0.12	0.15	0.12	0.09	0.09	0.07	0.07
	-F_1	-	0.29	0.24	0.28	0.21	0.13	0.12	0.10	0.10
Exch. Ca (meq/100 g)										
-with cassava	-F_0	0.33	0.43	0.37	0.38	0.29	0.30	0.29	0.29	0.34
	-F_1	0.62	0.72	0.87	0.94	0.52	0.61	0.59	0.54	0.48
-without cassava	-F_0	-	0.38	0.34	0.37	0.28	0.28	0.26	0.25	0.29
	-F_1	-	0.84	0.79	0.98	0.62	0.63	0.62	0.54	0.39
Exch. Mg (meq/100 g)										
-with cassava	-F_0	0.14	0.19	0.15	0.16	0.11	0.15	0.14	0.11	0.13
	-F_1	0.22	0.24	0.29	0.31	0.16	0.21	0.21	0.19	0.17
-without cassava	-F_0	-	0.14	0.13	0.15	0.11	0.13	0.12	0.10	0.11
	-F_1	-	0.28	0.26	0.31	0.21	0.20	0.22	0.17	0.13
Exch. Al (meq/100 g)										
-with cassava	-F_0	2.0	2.0	1.9	2.1	2.0	2.0	2.0	1.9	1.7
	-F_1	1.7	1.6	1.4	1.3	1.7	1.8	1.6	1.7	1.9
-without cassava	-F_0	-	2.0	2.1	2.0	2.1	2.1	2.1	2.2	2.0
	-F_1	-	1.5	1.6	1.3	1.6	1.8	1.7	1.9	1.9
Al-saturation (%)										
-with cassava	-F_0	78	72	70	74	79	78	78	79	75
	-F_1	61	55	49	47	66	65	62	66	73
-without cassava	-F_0	-	74	77	75	80	80	81	82	80
	-F_1	-	51	55	45	61	64	66	69	75

[1] No data for the 2d MAP

[2] Data are average values for with and without windbreaks

e. Change in total root length and mycorrhizal infection

Figure 22 shows the effect of the dry and wet seasons on total root length, on vesicular arbuscular mycorrhiza, VAM-infection and spore numbers. Root length in the top 20 cm of soil decreased during the dry season, while the percent VAM infection initially increased up to the third month and then decreased due to drought. Spore numbers, on the other hand, increased markedly during the dry season. During the first two months of rain, root length in the top 20 cm of soil increased from 1.2 to 37 meters/plant, after which it decreased markedly again. At the same time, the percentage of root infection increased from 5 to nearly 25%. Thus, at the end of May plants had on average about 7.5 meters of VAM infected roots. This increase in root infection was accompanied by a marked decrease in spore numbers as spores germinated in response to increased root activity. Following this flush of top and root growth at the beginning of the rainy season, root growth and VAM infection decreased, while spore numbers again increased. Towards the end of the rainy season root length stabilized while percentage of infection again increased.

SUMMARY AND CONCLUSIONS

The data presented clearly indicate that cassava growth is highly dependent on climatic conditions, mainly temperature and rainfall, as well as on the soil fertility conditions. While cassava can grow and produce reasonably well in low fertility soils, under those conditions the crop is highly responsive to the application of chemical fertilizers. In the four trials at the two locations in Colombia, fertilizer application increased root yields on average 56% over the unfertilized check. Fertilization also had a major effect on most other growth parameters measured, such as total DM production, DM in roots, stems, leaves and fallen leaves, as well as the absorption of all major and secondary nutrients (**Table 27).** Concentrations of nutrients in the various plant tissues also tended to be higher in fertilized than in unfertilized plants, even of those nutrients not applied in the fertilizer. But nutrient concentrations varied markedly between the various tissues, the location of those tissues in the plant (upper, middle or lower part), and in general tended to decrease with the age of the plant. However, this was also very dependent on the climatic conditions during the growth cycle: nutrient concentrations tended to decrease during periods of drought and increase during the onset of rains when plants had a flush of new leaves and rapid growth. To diagnose nutritional problems it is therefore very important to analyze a defined indicator tissue at a specific time in the growth cycle. In most cases, for cassava the best indicator tissue is the youngest fully expanded leaf (YFEL) blade collected at 3-4 months after planting. However, if this happens to be during a long and severe drought it is better to wait for the next wet season and sample the same indicator tissue about 2-3 months after the onset of rains. The nutrient concentrations in the YFEL blades determined in this manner can be compared with the tables of critical nutrient concentrations in cassava plant tissue, or with the nutrient concentrations corresponding to specific nutritional states of the plant (Howeler, 2002). A correct diagnosis of nutritional problems is the first and most important step in determining the most suitable way to supply the correct fertilizers to obtain and maintain high yields of cassava.

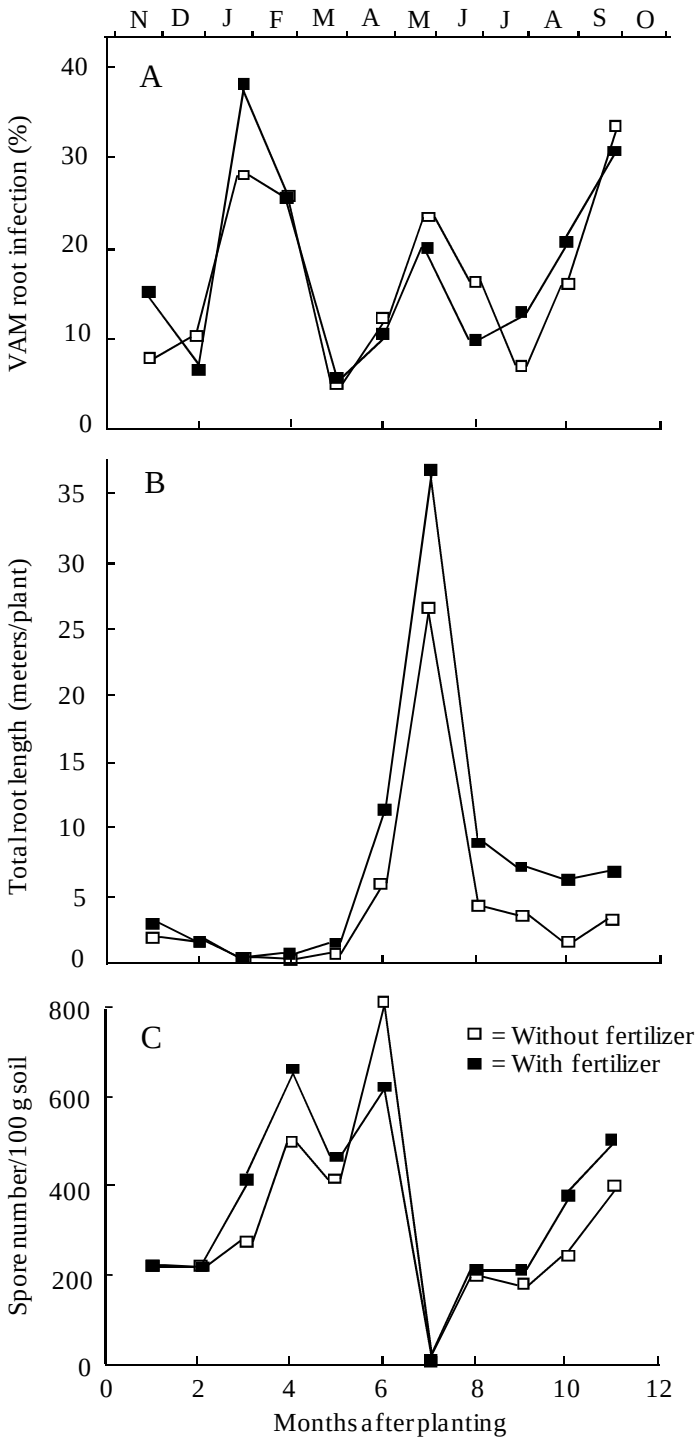

Figure 22. Change in VA-mycorrhizal (VAM) root infection (A) and total root length in the top 20 cm of soil (B) of cassava, MVen 77, and the VAM spore numbers in soil (C) during an 11-month growth cycle in fertilized and unfertilized plots in Carimagua, in 1984/85. Data are the average of plots with and without elephant grass windbreaks

Table 27. Summary and comparison of the most pertinent data from five experiments on the dry matter and nutrient accumulation in different plant parts at time of harvest of cassava.

	Indonesia		Quilichao				Carimagua			
	Varieties		1978/79[1]		1982/83[2]		1983/84[3]		1984/85[4]	
Parameters	Mangi	SPP	F_0[5]	F_1[5]	F_0	F_1	F_0	F_1	F_0	F_1
Fresh root yield (t/ha)	53	65	34	52	17	22	24	32	11	23
Total DM production (g/plant)	3,773	3,384	1,167	1,925	631	865	1,115	1,456	521	1,164
Total DM in roots (g/plant)	2,132	2,250	811	1,387	439	561	688	894	282	585
Total DM in fallen leaves (g/plant)	-	-	-	-	94	135	99	119	86	159
Total DM production (t/ha)	44.6	40.0	18.2	30.1	9.9	13.5	17.4	22.7	8.1	18.2
Total DM in roots (t/ha)	25.2	26.6	12.7	21.6	6.8	8.8	10.8	14.0	4.4	9.1
Total DM in stems (t/ha)	18.4	12.6	4.7	7.2	1.4	2.4	4.5	5.8	2.0	6.1
Total DM in leaves (t/ha)	1.0	0.8	0.9	1.2	0.1	0.2	0.7	1.1	0.4	0.5
DM content of leaves (%)	21.8	22.0	37.3	31.7	-	-	-	-	-	-
DM content of stems (%)	30.5	26.3	25.5	23.9	-	-	-	-	-	-
DM content of roots (%)	41.0	34.8	30.8	41.6	-	-	-	-	-	-
Total absorption of N (kg/ha)	132	124	209	315	124	164	123	197	66	140
Total absorption of P (kg/ha)	48	45	18	37	8	14	16	30	8	23
Total absorption of K (kg/ha)	477	487	144	237	48	67	92	184	48	122
Total absorption of Ca (kg/ha)	166	155	71	77	46	67	67	102	29	63
Total absorption of Mg (kg/ha)	53	43	25	32	17	21	27	28	9	19
Total N in roots (kg/ha)	38	45	101	152	67	70	30	67	14	33
Total P in roots (kg/ha)	28	28	10	22	5	8	8	17	4	11
Total K in roots (kg/ha)	268	317	90	162	33	42	55	102	25	66
Total Ca in roots (kg/ha)	34	51	24	20	10	18	5	15	2	7
Total Mg in roots (kg/ha)	20	18	9	11	6	6	6	8	3	5
N conc. in upper leaves at 2-4 MAP			5.06	5.73	4.87	5.12	4.57	5.19	4.47	4.74
P conc. in upper leaves at 2-4 MAP			0.31	0.38	0.35	0.39	0.34	0.38	0.30	0.34
K conc. in upper leaves at 2-4 MAP			1.72	1.85	1.59	1.68	1.29	1.61	1.08	1.06
Ca conc. in upper leaves at 2-4 MAP			0.59	0.57	0.80	0.83	0.68	0.76	0.50	0.52
Mg conc. in upper leaves at 2-4 MAP			0.31	0.31	0.31	0.34	0.25	0.28	0.25	0.28
S conc. in upper leaves at 2-4 MAP			0.30	0.31	0.37	0.37	0.29	0.30	0.29	0.26
N concentration in leaves at harvest	2.56	2.34								
N concentration in stems at harvest	0.37	0.48								
N concentration in roots at harvest	0.15	0.17								
P concentration in leaves at harvest	0.24	0.23								
P concentration in stems at harvest	0.10	0.12								
P concentration in roots at harvest	0.11	0.11								
K concentration in leaves at harvest	1.59	1.33								
K concentration in stems at harvest	1.05	1.26								
K concentration in roots at harvest	1.06	1.19								

[1] data for MCol 22; [2] data include fallen leaves

[3] data from plots without irrigation; [4] data from plots with elephant grass wind breaks

[5] F_0 = unfertilized, F_1 = fertilized

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CHAPTER 15

EFFECT OF CASSAVA PRODUCTION ON SOIL FERTILITY AND THE LONG-TERM FERTILIZER REQUIREMENTS TO MAINTAIN HIGH YIELDS

Reinhardt Howeler[1]

INTRODUCTION

Cassava has the reputation to cause serious soil degradation due to excessive uptake of nutrients leading to soil nutrient depletion, or by causing serious soil erosion when grown on slopes. Is this perception correct and based on any scientific evidence? Or is it based on the simple observation that cassava is oftentimes grown on degraded soils, and mainly by poor farmers trying to make a living on the poorest of soils? Thus, the question is whether the planting of cassava is the cause or the consequence of soil degradation.

This chapter examines whether cassava does indeed extract and remove more nutrients from the soil than other crops, and what effect this has on soil fertility. It also describes how better management of the crop can reduce nutrient depletion and maintain high yields when cassava is grown continuously for many years on the same soil.

Evidence of Soil Degradation

Besides the simple observation that cassava is often grown on degraded soil, there is also good scientific evidence that continued cassava cultivation can have a detrimental effect on the soil's chemical and physical characteristics. **Table 1** shows the effect of land use for different crops on the fertility status of Haplic Acrisols in southern Vietnam. Comparing the long-term effect of forest, rubber, cashew, sugarcane and cassava on the soil's chemical and physical properties, Cong Doan Sat and Deturck (1998) reported that long-term cassava cultivation caused the most serious reduction in the organic C and total N content of the soil, as well as that of the CEC and K and Mg status; however, cassava had increased the levels of available P as compared to forest or cashew, presumably due to some P-fertilizer application. Cassava also caused the soils to have a low clay content, low aggregate stability and a low volumetric water content and infiltration rate. However, the evidence that cassava "caused" these negative changes is not conclusive, because heavier and more fertile soils with greater aggregate stability are generally used for higher value crops like rubber, cashew and sugarcane. Moreover, cassava, an annual crop, is compared mostly with perennial tree crops and sugarcane; the latter generally receives much higher fertilizer rates than cassava and may be ratooned for two or more years before the soil needs to be prepared again. Cassava should have been compared with other annual crops as it is mainly the need for frequent soil preparation that causes the marked reduction in organic matter (OM) content.

Other "evidence" that cassava cultivation causes soil degradation is shown in **Figure 1**, which indicates that continued cassava cultivation over 30 years without fertilizer application resulted in ever lower cassava yields in three soil series in Thailand. However,

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similar or worse yield declines could be expected if other annual food crops were grown without fertilizers, due to the inevitable nutrient depletion by removal of harvested products. An example of this is shown in **Figure 2** which indicates that continuous cropping of upland rice without fertilizers in Vietnam resulted in zero yield in the fourth crop cycle, while cassava still produced about 40% of its first year yield after four years of cropping at the same site (Nguyen Tu Siem, 1992).

Table 1. Chemical properties of various horizons of Haplic Acrisols that had been under different land use for many years in southeastern Vietnam.

	Forest	Rubber	Cashew	Sugarcane	Cassava	CV (%)
Organic C (%)	1.032 a	0.839 ab	0.579 ab	0.796 ab	0.496 b	44.7
Total N (%)	0.058 a	0.054 ab	0.032 bc	0.040 abc	0.022 c	36.7
Available P (Bray II) (ppm)						
-1st horizon	5.21 b	20.90 a	4.85 b	20.68 a	15.33 ab	37.5
-2nd horizon	2.48 b	7.03 a	3.19 b	7.92 a	5.31 ab	32.6
-3rd horizon	1.57 b	2.83 ab	1.08 ab	3.82 a	3.82 a	44.6
CEC (meq/100 g)	3.43 a	2.94 a	2.39 ab	3.24 a	1.53 b	27.1
Exch. K (meq/100 g)						
-1st horizon	0.132 a	0.127 a	0.070 ab	0.051 b	0.060 b	66.3
-2nd horizon	0.073 a	0.046 ab	0.031 ab	0.022 b	0.021 b	75.1
Exch. Mg (meq/100 g)	0.145 a	0.157 a	0.046 ab	0.055 ab	0.036 b	89.1

Values are average of 6-10 profiles per cropping system. Within rows data followed by the same letter are not significantly different at 5% level by Tukey's Studentized Range Test.
Source: *Cong Doan Sat and Deturck, 1998.*

While there is no doubt that continuous cassava cultivation without nutrient inputs will deplete the soil's nutrient status, as nutrients are removed from the field in the harvested products, this is also true for other crops. However, if adequate amounts of nutrients are applied annually to compensate for this nutrient removal, then the fertility status of the soil can be maintained.

Nutrient Extraction by Cassava as Compared to That of Other Crops.

Since cassava root yields are often an order of magnitude higher than those of the cereals and grain legumes, many people assume that cassava roots must extract and remove a large amount of nutrients. However, cassava roots contain about 60-70% water and the rest is mostly starch and some fiber, but with very little N (2-3% protein) and other plant nutrients.

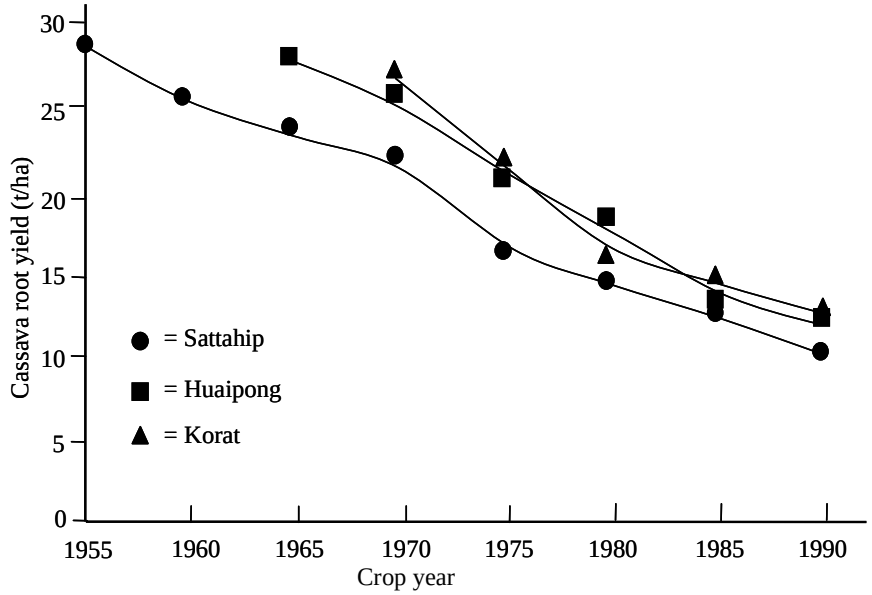

Figure 1. Decline in fresh root yields due to continuous cultivation without fertilizers in three soil series in Thailand.
***Source**: Sittibusaya, 1993.*

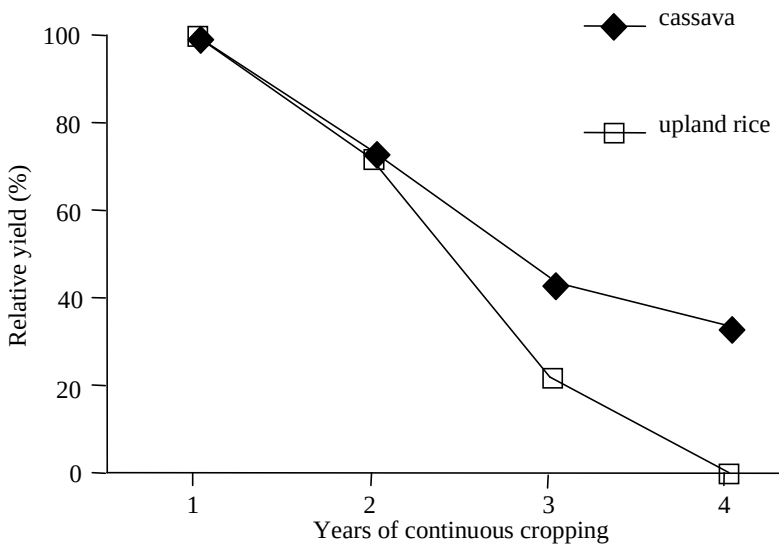

Figure 2. Yield reduction of upland rice and cassava due to fertility decline as a result of continuous cropping without fertilizer application. 100% corresponds to 18.9 t/ha for fresh cassava roots and 2.55 t/ha of rice.
***Source**: adapted from Nguyen Tu Siem, 1992.*

Table 2 shows the yields and nutrient contents in the harvested products of cassava and several other crops as reported in the literature. In spite of a high yield of cassava, the removal of N and P was lower than for most other crops, while that of K was higher than most other crops but similar to tobacco, sugarcane and sweet potato. The table also shows that per ton of dry matter (DM) produced cassava removes in the root harvest much less N and P, and less K than most other crops.

Similar data were also reported by Amarasiri and Perera (1975) for several crops grown in Sri Lanka. **Table 3** shows that in that case cassava yields were exceptionally high at 45 t/ha, resulting in high levels of nutrient absorption, both in the roots as well as in the whole plant. N and P contents of the cassava roots were similar to the N and P contents of the harvested products of most other crops, but the K content of the cassava roots were much higher than those of the harvested products of all other crops. Thus, N and P removal by the harvest of cassava roots is generally lower, but the K removal in the roots can be much higher than that removed by the growing of other crops.

Table 2. Average nutrient removal in the harvested products of cassava and various other crops, expressed in both kg/ha and kg/t DM produced, as reported in the literature.

	Yield (t/ha)		(kg/ha)			(kg/t DM produced)		
Crop/plant part	fresh	dry[1)]	N	P	K	N	P	K
Cassava/fresh roots	35.7	13.53	55	13.2	112	4.5	0.83	6.6
Sweet potato/fresh roots	25.2	5.05	61	13.3	97	12.0	2.63	19.2
Maize/dry grain	6.5	5.56	96	17.4	26	17.3	3.13	4.7
Rice/dry grain	4.6	3.97	60	7.5	13	17.1	2.40	4.1
Wheat/dry grain	2.7	2.32	56	12.0	13	24.1	5.17	5.6
Sorghum/dry grain	3.6	3.10	134	29.0	29	43.3	9.40	9.4
Beans[2)]/dry grain	1.1	0.94	37	3.6	22	39.6	3.83	23.4
Soya/dry grain	1.0	0.86	60	15.3	67	69.8	17.79	77.9
Groundnut/dry pod	1.5	1.29	105	6.5	35	81.4	5.04	27.1
Sugarcane/fresh cane	75.2	19.55	43	20.2	96	2.3	0.91	4.4
Tobacco/dry leaves	2.5	2.10	52	6.1	105	24.8	2.90	50.0

[1)] Assuming cassava to have 38% DM, grain 86%, sweet potato 20%, sugarcane 26%, dry tobacco leaves 84%.

Source: *Howeler, 1991.*

Table 4 shows that at time of harvest of cassava, most DM (about 60%) is in the roots, but most N, P, Ca, Mg, S and micro-nutrients are in the leaves and stem, which are generally returned to the soil. Only in case of K, the removal in roots is higher than that returned in tops and fallen leaves, i.e. about 60 and 40%, respectively. Naturally, when all plant residues are removed from the field for feeding animals, or are used as fuel in the kitchen, the removal of nutrients increases substantially, especially that of N as well as Ca and Mg, which are concentrated in the leaves and stems, respectively (Howeler, 1985a).

Table 3. Yield and nutrient removal of several crops grown on an Alfisol in Sri Lanka.

Crop (duration in days)	Plant part	Yield (t/ha)	Nutrients absorbed (t/ha) N	P	K	Ca	Mg	S
Cassava (180)	Fresh roots	45	62	10	164	12	22	3
(Manihot esculenta)	Total plant		202	32	286	131	108	15
Sweet potato (100)	Fresh roots	15	31	6	51	10	4	3
(Ipomoea batatas)	Total plant		89	17	187	44	26	14
Rice (130)	Grain	5	58	12	10	2	7	3
(Oryza sativa)	Total plant		100	18	151	27	23	9
Sorghum (100)	Grain	4	68	8	16	3	6	2
(Sorghum vulgaris)	Total plant		101	13	108	17	14	5
Maize (105)	Grain	4	64	7	13	2	2	6
(Zea maiz L.*)*	Total plant		118	11	155	32	25	13
Cotton (90)	Seed cotton	1.9	40	6	7	6	5	2
(Gossypium sp.*)*	Total plant		77	14	68	34	21	19
Cowpea (90)	Grain	1.5	50	4	19	3	2	2
(Vigna unguiculata)	Total plant		60	5	36	11	6	4
Groundnut (100)	Grain	1.8	88	5	12	1	3	2
(Arachis hypogea)	Total plant		101	6	34	12	8	4
Soybean (90)	Grain	1.2	103	10	34	6	4	3
(Glycine max)	Total plant		118	11	47	16	9	5

Source: *Amarasiri and Perera, 1975.*

Table 5 shows the DM production, nutrient uptake and removal of seven crops grown for 22 months in the same experiment in Sri Racha, Thailand. In case of cassava for root production, the total nutrient uptake per ha was similar to that of maize, sorghum, peanut, mungbean and pineapple, but the removal of N, P and Mg in the harvested roots was lower than in the harvested products of any other crop, while the removal of K and Ca was similar to that of other crops but much lower than pineapple (Putthacharoen *et al*., 1998). However, when cassava was grown for forage production, with frequent cutting and removal of whole tops, the nutrient removal was higher than that of any other crop. This management system is highly productive, but also requires large inputs of chemical fertilizers to prevent soil nutrient depletion (Martwanna *et al*., 2009).

As cassava root yields increase due to fertilization or more favorable growing conditions, the nutrient removal in both roots and in the total biomass also increases (**Table 4).** This is also clearly shown by the data in **Table 6**, which shows the yields of cassava roots and whole plants, as well as the nutrient contents in both, as reported in the literature. In this case fresh root yields varied from only 6 to as high as 65 t/ha. Based on the data set

of the 15 sources in **Table 6** that show both the fresh and dry root yields, the average nutrient removal in an "average" root yield of 28.9 t/ha is about 67.1 kg N, 11.2 kg P and 88.1 kg K/ha, while that by the whole plant would be 179.5 kg N, 22.7 kg P and 156.1 kg K/ha (**Table 7**). This latter table also shows the average nutrient removal per ton of fresh and dry roots. However, when the reported N, P and K removal data in **Table 6** are plotted against fresh root yields, we see that this is not a linear relationship (**Figure 3**). As yields increase, due to better growing conditions, the nutrient concentrations in the plant tissues also tend to increase, resulting in a curvilinear relationship between nutrient removal and yield. Thus, at a fresh root yield of 15 t/ha the nutrient removal in the roots is only about 30 kg N, 3.5 kg P and 20 kg K/ha rather than 34.8 kg N, 5.85 kg P and 45.75 kg K as predicted from the average values shown in **Table 7**. Thus, at the relatively low yields that farmers usually obtain, the nutrient removal in the harvested roots is actually quite low, especially of P, as long as crop residues are returned to the soil. However, in order to maintain or increase their yields, farmers may have to apply chemical fertilizers or manures, or they may plant green manures, depending on the native fertility of the soil.

Table 4. Dry matter and nutrient distribution in 12-month-old cassava cv. M Ven 77, grown with and without fertilization in Carimagua, Colombia in 1983/84.

	DM	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	(t/ha))	(kg/ha)										
Unfertilized												
-tops	5.11	69.1	7.4	33.6	37.4	16.2	8.2	0.07	0.03	0.45	0.33	0.26
-roots	10.75	30.3	7.5	54.9	5.4	6.5	3.3	0.08	0.02	0.38	0.02	0.10
-fallen leaves	1.55	23.7	1.5	4.0	24.7	4.0	2.5	0.04	0.01	-	0.37	0.18
Total	**17.41**	**123.1**	**16.4**	**92.5**	**67.5**	**26.7**	**14.0**	**0.19**	**0.06**	**-**	**0.72**	**0.54**
Fertilized												
-tops	6.91	99.9	11.7	74.3	55.0	15.3	9.6	0.08	0.03	0.78	0.57	0.30
-roots	13.97	67.3	16.8	102.1	15.5	8.4	7.0	0.07	0.03	0.90	0.06	0.17
-fallen leaves	1.86	30.5	2.0	7.1	31.9	4.7	2.6	0.05	0.02	-	0.46	0.19
Total	**22.74**	**197.7**	**30.5**	**183.5**	**102.4**	**28.4**	**19.3**	**0.20**	**0.08**	**-**	**1.09**	**0.66**

Source: *Howeler, 1985a.*

The large extraction of K in each root harvest can lead to K exhaustion of the soil. Thus, den Doop (1937) reported that in three consecutive cassava plantings without applied K, yields decreased from 15 t/ha in the first year to 4 t/ha in the third year. Similarly, Chan (1980) reported that in a long-term fertility trial on mineral soils in Malaysia yields decreased from 32 to 20 t/ha in nine consecutive cassava crop cycles without fertilization; with application of 112 kg N, 68 kg P and 156 kg K/ha yields actually increased from 30 to 54 t/ha in the ninth crop. The yield decline without fertilization was mainly due to K exhaustion.

Table 5. Total dry matter (DM) production and nutrient uptake (A), nutrients removed (B) and DM and nutrients returned to the soil (C) of seven crops grown during 22 months in Sri Racha Research Station, Sri Racha, Thailand, from 1989 to 1991.

Crop	DM	N	P	K	Ca	Mg
A. Total dry matter produced and nutrient uptake (kg/ha)						
Cassava for roots	14,920	284	39	192	167	42
Cassava for forage	17,186	380	47	256	186	67
Maize	21,538	219	57	357	40	39
Sorghum	22,222	225	52	355	61	46
Peanut	13,489	347	31	236	93	36
Mungbean	5,990	171	21	128	60	25
Pineapple	26,761	243	46	465	136	43
F-test	**	**	**	**	**	**
CV (%)	12.24	11.21	19.10	14.69	15.66	12.20
LSD (P<0.01)	5.081	72.5	19.4	100.6	39.4	11.9
B. Dry matter and nutrients removed from the field in the harvested products (kg/ha)						
Cassava for roots	5,185	48	7	60	14	6
Cassava for forage	15,695	363	43	240	162	62
Maize	8,782	118	44	87	6	11
Sorghum	5,097	79	25	51	10	9
Peanut	4,899	213	19	53	6	8
Mungbean	2,878	117	15	62	9	11
Pineapple	7,582	83	15	190	51	19
C. Dry matter and nutrients returned to the soil in the non-harvested products (kg/ha)						
Cassava for roots	9,735	236	46	132	154	35
Cassava for forage	1,491	17	4	16	24	5
Maize	12,756	101	13	269	34	28
Sorghum	17,125	147	27	304	51	37
Peanut	8,590	133	12	183	87	28
Mungbean	3,112	54	7	66	51	14
Pineapple	19,179	160	31	176	85	24

Source: *Putthacharoen et al., 1998.*

Similar results were reported by Kabeerathumma *et al*. (1990) for a long-term NPK trial conducted in Trivandrum, Kerala, India. After ten years of continuous cassava cropping the yields without K application had decreased from 22 t/ha in the first year to about 6 t/ha in the tenth year. In the treatment without K the exchangeable K in the soil had decreased from an initial level of 0.17 to only 0.07 meq/100 g, indicating a clear depletion of the K status of the soil due to repeated cassava cropping and harvests; with K application the exchangeable K level had increased to 0.23 meq/100 g and yields had increased to 28 t/ha **(Figure 4)**.

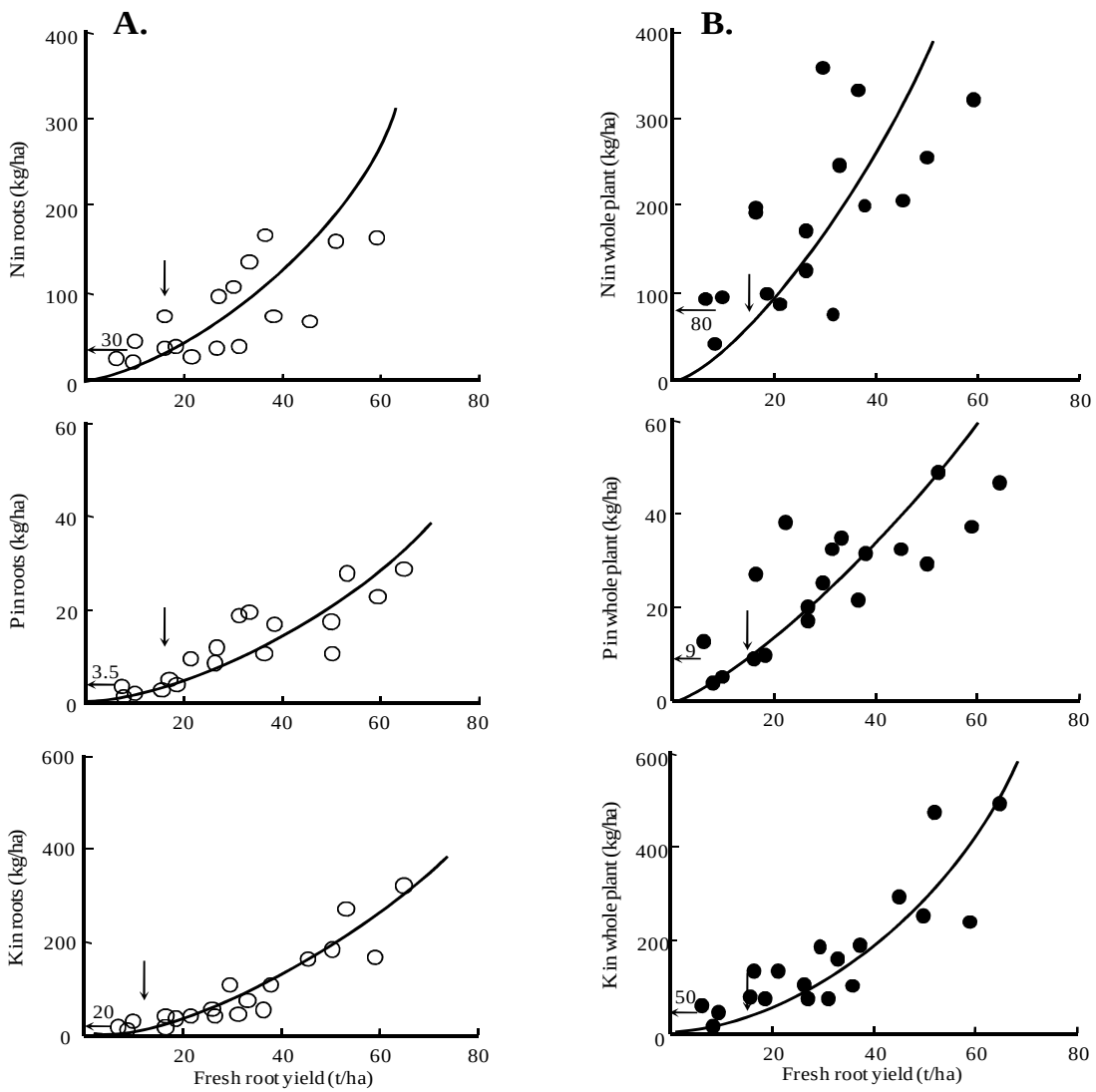

Figure 3. Relation between the amounts of N, P and K in cassava roots (A) or in the whole plant (B) and the fresh root yield, as reported by various sources in the literature. Arrows indicate the approximate nutrient contents corresponding to a fresh root yield of 15 t/ha.
***Source**: Howeler, 2002; 2004.*

Table 6. Fresh and dry yield, as well as nutrient content in cassava roots and in the whole plant at time of harvest, as reported in the literature.

	Yield (t/ha)		Nutrient content (kg/ha)					
Plant part	fresh	dry	N	P	K	Ca	Mg	Source/cultivar
Roots	6.0	1.52	18	2.2	15	5	2	Putthacharoen *et al.*, 1998
Whole plant	-	4.37	91	12.2	55	46	15	1989/90 Rayong 1
Roots	8.7	2.68	13	0.9	4	3	2	Sittibusaya (unpublished)
Whole plant	-	4.23	39	3.2	10	21	8	unfertilized Rayong 1
Roots	~9.0	3.24	37	1.5	23	4	2	Paula *et al*., 1983
Whole plant	-	6.54	93	4.0	40	30	9	unfertilized Branca St. Cat.
Roots	~15.9	5.58	66	2.7	17	8	5	Paula et al., 1983
hole plant	-	10.62	197	8.1	61	100	20	unfertilized Riqueza
Roots	16.1	3.64	30	4.7	45	9	5	Putthacharoen et al., 1998
Whole plant	-	10.55	193	27.0	137	122	27	1990/91 Rayong 1
Roots	18.3	5.52	32	3.6	35	5	4	Sittibusaya (unpublished)
Whole plant	-	9.01	95	9.9	65	37	15	fertilized Rayong 1
Roots	21.0	-	21	9.2	44	8	10	Kanapathy, 1974
Whole plant	-	-	86	37.2	135	45	34	Malaysia, peat soil
Roots	26.0	10.75	30	8.0	55	5	7	Howeler, 1985a
Whole plant	-	17.41	123	16.0	92	67	27	unfertilized MVen 77
Roots	26.6	12.81	91	11.3	47	5	6	Cadavid, 1988
Whole plant	-	19.10	167	19.1	76	32	19	unfertilized CM523-7
Roots	~28.5	10.28	100	8.7	107	15	13	Paula et al., 1983
Whole plant	-	19.56	353	24.8	174	133	37	fertilized Riqueza
Roots	31.0	-	31	18.9	47	-	-	Sittibusaya and
Whole plant	-	-	73	31.9	72	-	-	Kurmarohita, 1978
Roots	32.3	15.39	127	19.1	71	6	5	Cadavid, 1988
Whole plant	-	25.04	243	34.4	147	56	25	fertilized CM523-7
Roots	~36.0	12.60	161	10.0	53	16	12	Paula et al., 1983
Whole plant	-	20.92	330	20.5	100	88	30	fertilized Branca St. Cat.
Roots	37.5	13.97	67	17.0	102	16	8	Howeler, 1985b
Whole plant	-	22.74	198	31.0	184	102	28	unfertilized MCol 22
Roots	45.0	-	62	10.0	164	12	22	Amarisisi and Pereira, 1975
Whole plant	-	-	202	32.0	286	131	108	Sri Lanka

Table 6. (continued)

Plant part	Yield (t/ha) fresh	dry	Nutrient content (kg/ha) N	P	K	Ca	Mg	Source/cultivar
Roots	50.0	-	153	17.0	185	25	6	Cours, 1953
Whole plant	-	-	253	28.0	250	42	29	Madagascar
Roots	52.7	25.21	38	27.9	268	34	19	Nijholt, 1935
Whole plant	111.1	44.65	132	48.5	476	161	52	cv. Manggi
Roots	59.0	21.67	152	22.0	163	20	11	Howeler and Cadavid, 1983
Whole plant	-	30.08	315	37.0	238	77	32	fertilized MCol 22
Roots	64.7	26.59	45	28.2	317	51	18	Nijholt, 1935
Whole plant	110.6	39.99	124	45.3	487	155	43	cv. Sao Pedro Preto
Roots	**30.8**	**-**	**67.0**	**11.7**	**92.7**	**-**	**-**	**Average 19 sources**
Whole plant	**-**	**-**	**174.0**	**24.7**	**162.4**	**-**	**-**	

Source: *Howeler, 2002.*

Table 7. Average fresh and dry root yield, as well as the amount of nutrients removed when cassava roots or the whole plant are harvested, based on data from the literature[1)].

Plant part	Yield (t/ha) fresh	dry	Nutrient removal N	P	K	Ca	Mg
			(kg/ha)				
Roots	28.87	11.43	67.1	11.2	88.1	13.5	7.9
Whole plant		18.99	179.5	22.7	156.1	81.8	25.8
			(kg/t fresh roots)				
Roots	28.87	11.43	2.32	0.39	3.05	0.47	0.27
Whole plant		18.99	6.22	0.79	5.41	2.83	0.89
			(kg/t dry roots)				
Roots	28.87	11.43	5.87	0.98	7.71	1.18	0.69
Whole plant		18.99	15.70	1.99	13.66	7.16	2.26

Data are average of 15 data sets which have yields reported in dry weight in Table 6.
Source: *Howeler et al., 2001b.*

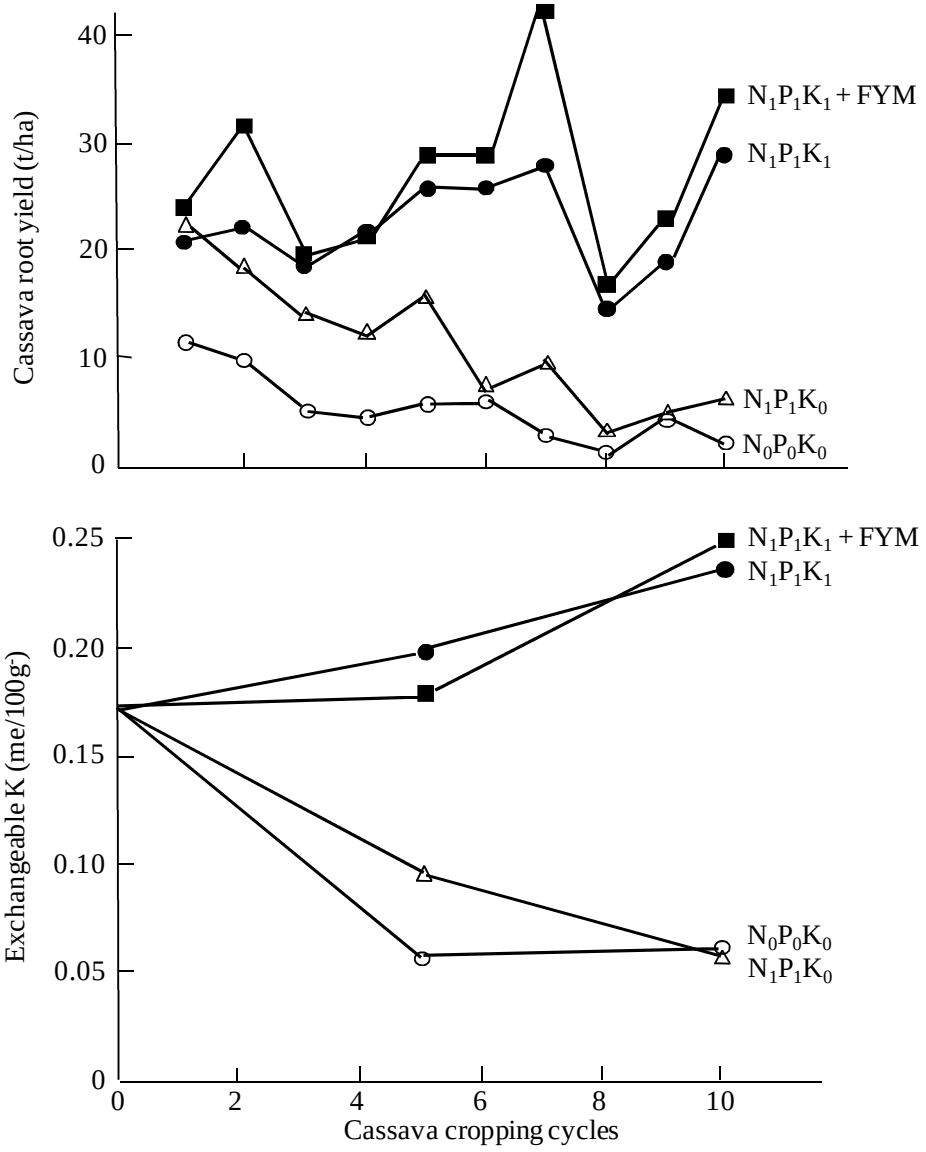

Figure 4. Cassava yield (top) and the exchangeable K content of the soil (bottom) during 10 years of continuous cropping with various NPK treatments in Trivandrum, Kerala, India.
Source: *Kabeerathumma et al., 1990.*

Very similar results were also reported by Howeler and Cadavid (1990) for a long-term NPK trial conducted for eight years in Quilichao, Colombia **(Figure 5)**. Root yields of about 30 t/ha could only be maintained with the application of 150 kg K/ha, which maintained the exchangeable K level of the soil at about 0.2 meq/100 g **(Figure 5B)**. Without K application yields slowly declined from 21 to 14 t/ha, while the exchangeable K content declined from 0.2 to 0.1 meq/100 g after eight cassava crop cycles.

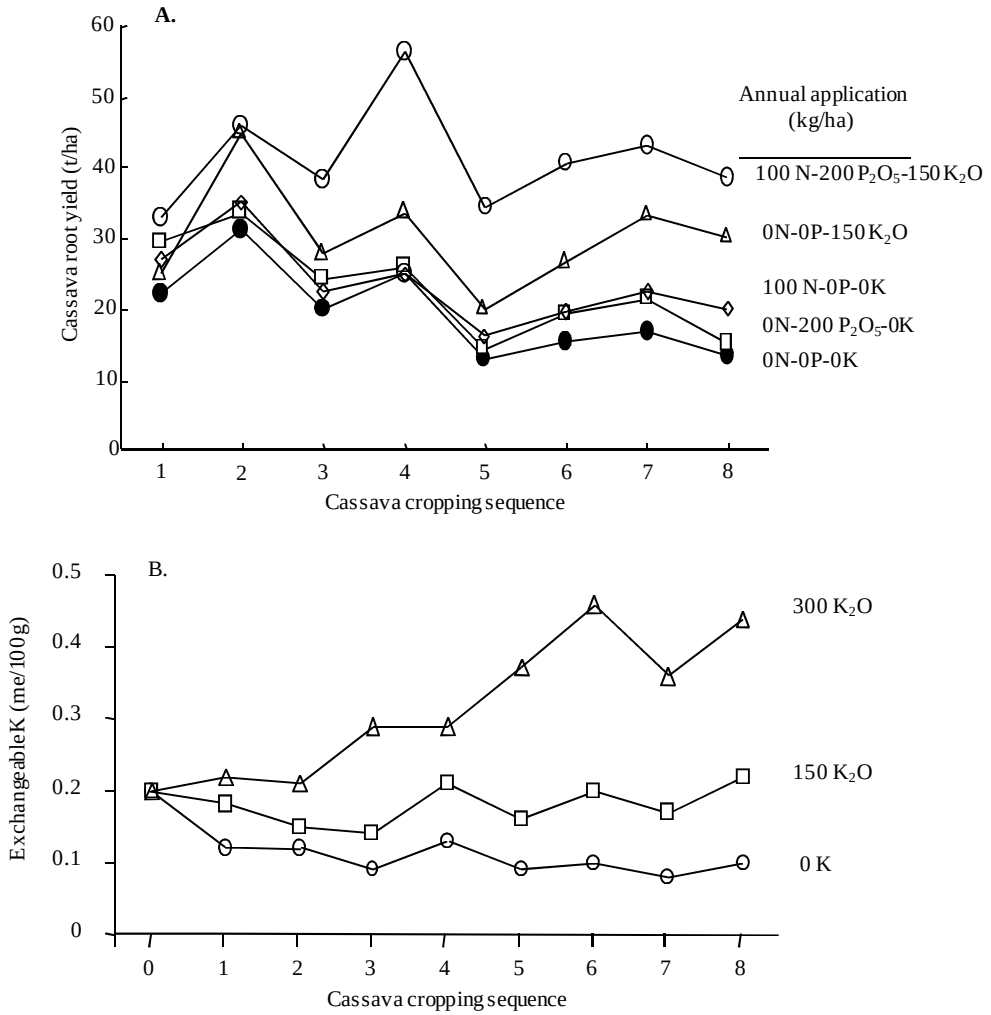

Figure 5. Effect of various levels of annual applications of N, P and K on cassava root yield (A), and on the exchangeable K content of the soil (B) during eight consecutive cropping cycles in a long-term NPK trial conducted at CIAT-Quilichao, Colombia.
Source: *Howeler and Cadavid, 1990.*

Many other long-term fertility trials have been conducted to determine the optimum amount and balance of N, P and K to maintain soil fertility and obtain high cassava yields (or total income when intercropped) for different types of soil. **Table 8** summarizes the results of 19 long-term fertility trials, conducted for 4 to 31 years of continuous cropping. The table shows that during the last year, K had become the most limiting nutrient in 12 trials, N in five trials and P in only two, as indicated by the low relative yields in plots where these nutrients had not been applied.

Table 8. Cassava root yield response to annual applications of various levels of NPK and the relative response[1)] to each nutrient during the last year of cropping in 16 long-term fertility trials conducted in Asia and Latin America.

Location	Varieties	No of years	Yield (t/ha)		Relative yields (%)		
			$N_0P_0K_0$	$N_2P_2K_2$	$N_0P_2K_2$	$N_2P_0K_2$	$N_2P_2K_0$
Bohol, Philippines	VC-1+Golden Yellow	4	7.5	20.4	58	84	33
Negros Oriental, Philippines	Lakan	4	7.1	13.9	71	129	76
Yogyakarta, Indonesia	Adira-1	4	6.2	10.9	60	87	81
Jatikerto, E. Java, Indonesia	Faroka	8	3.1	11.3	31	72	81
GSCRI, Nanning, China	SC201+SC205	8	12.9	18.6	70	82	85
Umas Jaya, Lampung, Indonesia	Adira-4	10	11.1	15.0	111	92	84
Serdang, Malaysia	Black Twig	10	20.7	51.0	69	72	57
Santander de Quilichao, Colombia	MCol 1684	13	12.9	30.0	94	96	71
	MCol 1684	11	12.2	30.7	64	92	42
Trivandrum, Kerala, India	H 1687	13	1.0	22.3	24	42	7
CATAS, Hainan, China	SC205+SC124	16	7.2	15.1	41	77	63
Tamanbogo, Lampung, Indonesia	Adira 4 [2)]	16	2.9	12.2	58	80	26
	Adira 4 [3)]	16	3.6	13.2	64	57	26
TNUAF, Thai Nguyen, Vietnam	KM60+Vinh Phu	17	4.4	21.8	67	71	16
Hung Loc ARC, Dong Nai, Vietnam	KM60 + SM937-26	20	6.8	20.1	70	81	24
Rayong FCRC, Thailand	Rayong 1	10	8.7	18.3	-	96	51
	Rayong 1 + Rayong 5	21	20.7	41.1	-	55	65
Khon Kaen FCRC, Thailand	Rayong 1 + R5	30	2.5	31.9	-	77	9
B Samrong FCRC, Thailand	Rayong 1 + R5	31	21.7	26.9	-	66	99

[1)] Yield in the treatment without the nutrient over the yield with the nutrient ($N_2P_2K_2$).
[2)] Monoculture; [3)] Intercropped with rice and maize

Figure 6 shows the importance of K in increasing both the root yield and the root starch content, while **Figure 7** shows that over time K became the most limiting nutrient when cassava was grown for many years on the same soil. The latter figure also shows that high root yields of 20-30 t/ha and a reasonable level of soil fertility could be maintained for at least 17 years of continuous cropping when medium levels of N, P and K were supplied annually.

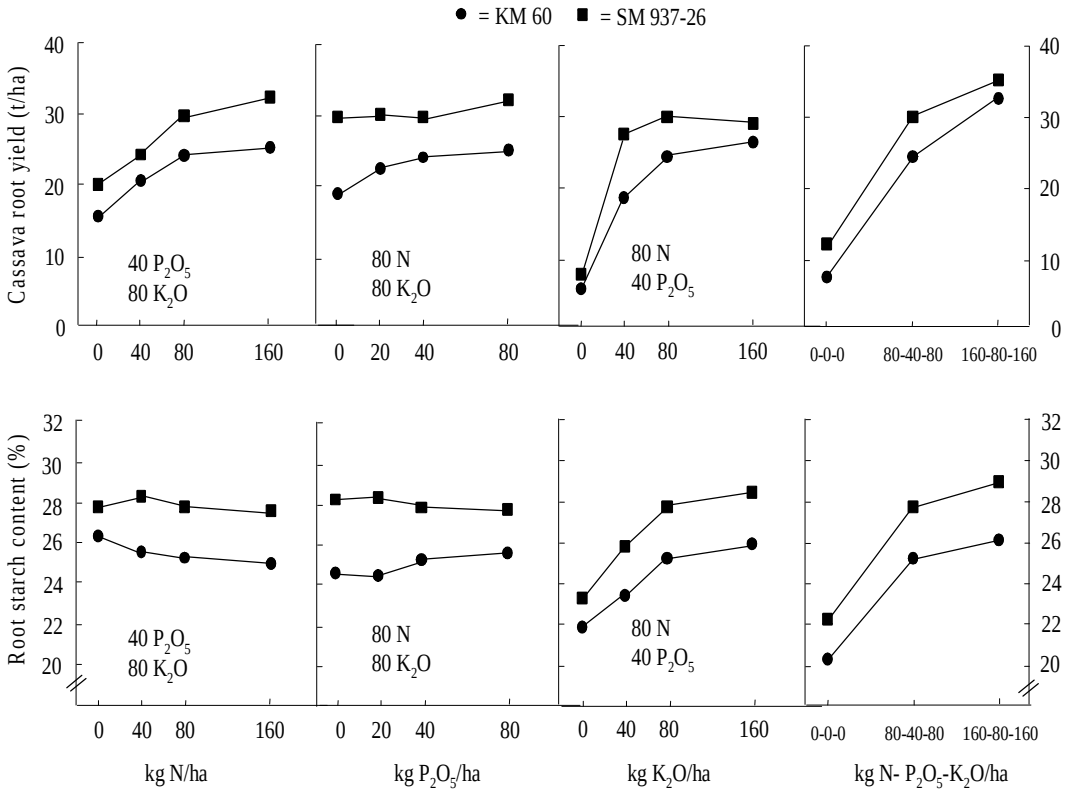

Figure 6. Effect of annual application of various levels of N, P and K on the root yield and starch content of two cassava varieties grown at Hung Loc Agriculture Research Center in Trang Bon district, Dong Nai, Vietnam in 2008/09 (19th year).

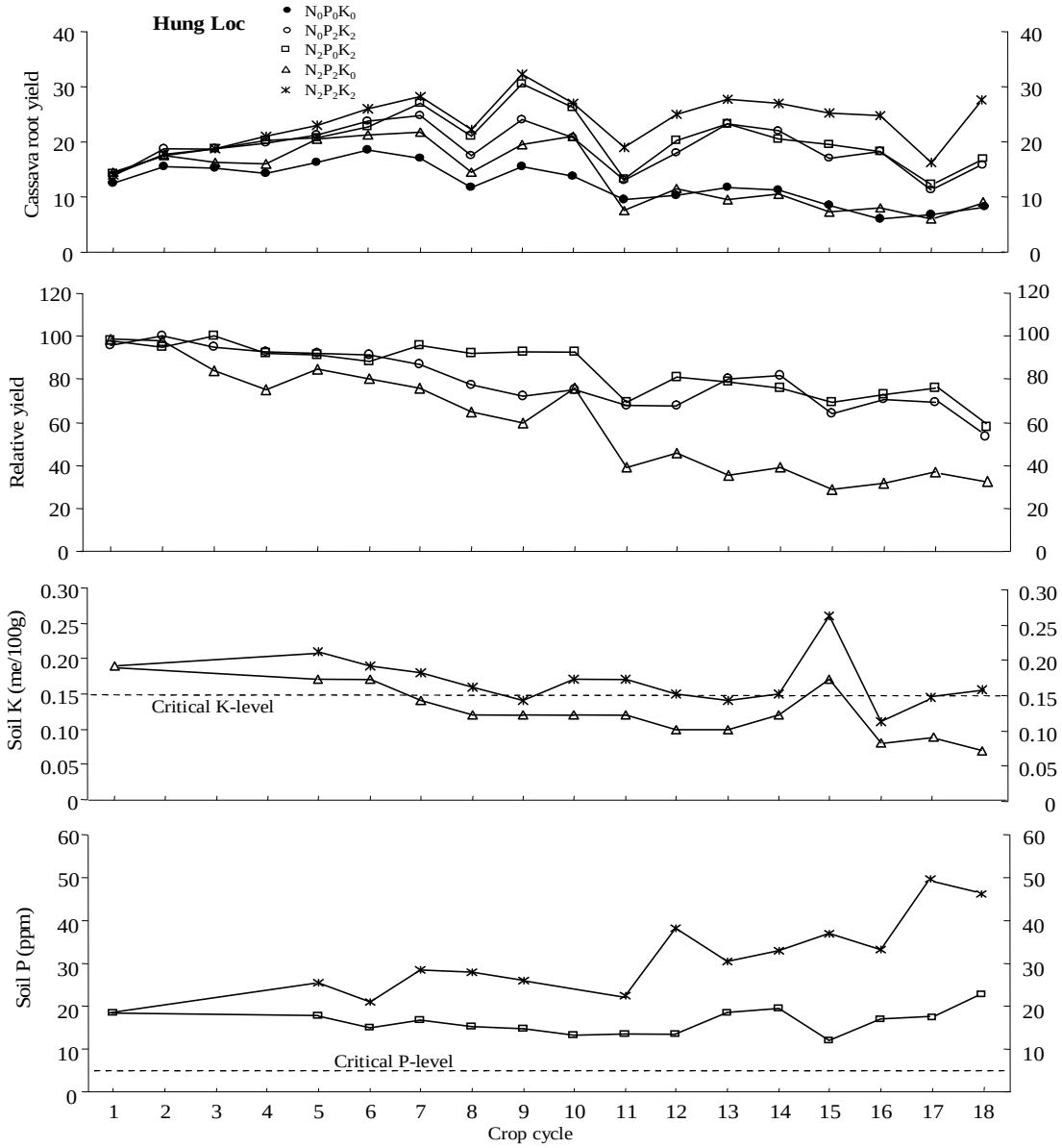

Figure 7. Effect of annual applications of N, P and K on cassava root yield, relative yield (yield without the nutrient over the highest yield with the nutrient) and the exchangeable K and available P (Bray 2) content of the soil during 18 years of continuous cropping in HungLoc Agric. Research Center in Dong Nai, Vietnam.

Source: *Nguyen Huu Hy, personal communication.*

Figure 8 shows that in Khon Kaen , Thailand, cassava could be grown for 25 years while maintaining high root yields of 30-40 t/ha when adequate amounts of NPK fertilizers (100 kg N + 50 P_2O_5 + 100 K_2O/ha) were applied annually and plant tops were incorporated

into the soil before each new planting. However, when no fertilizers were applied and plant tops were removed from the field, cassava yields dropped from about 30 t/ha in the first year to 7 t/ha in the sixth year of cropping due to nutrient depletion, especially that of K. Incorporation of plant tops, or the application of compost, in combination with chemical fertilizers, produced the highest yields.

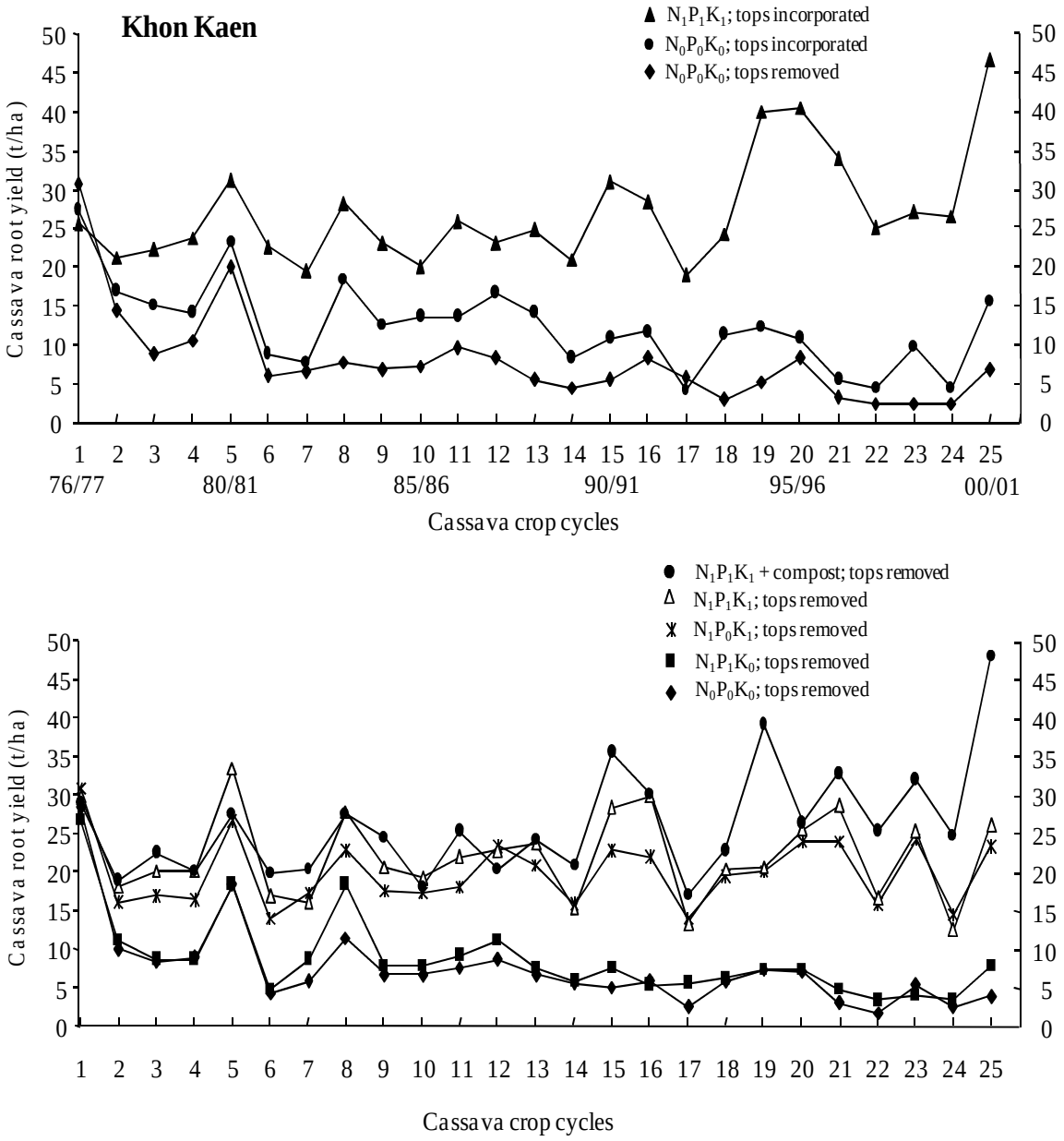

Figure 8. Effect of annual fertilizer application and crop residue management on cassava yields during 25 consecutive crops grown at Khon Kaen Field Crops Research Institute, Khon Kaen, Thailand.
***Source**: Chumpol Nakviroj and Kobkiet Paisancharoen, personal communication.*

CONCLUSIONS

1. While it is generally believed that cassava production degrades the soil because the crop extracts large amounts of nutrients from the soil, this is only the case if root yields are exceptionally high or when the leaves and stems are also harvested and removed from the field. If only roots are harvested, the removal of N and P is generally much lower than that of other crops, while the removal of K may be similar or slightly higher than that of other crops.
2. Nutrient removal in the root harvest is generally in the following order: K>N>P>Ca>Mg>S, while in the harvest of all plant parts this will probably be: N>K>Ca>Mg>P>S
3. As the nutrient status of the soil increases, this will not only tend to increase yields, but also the concentration of the nutrients in all plant tissues. As a result, the removal of nutrients in the harvest of roots or the whole plant increases exponentially as root yields increase.
4. The response of cassava to the application of N, P or K during the first few years of cropping will generally depend on the native fertility of the soil. However, due to the relatively large extraction and removal of K in each root harvest, the continuous cropping of cassava for many years on the same soil will almost always lead to a depletion of soil K. As such, K becomes the main limiting nutrient, followed by N and P.

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CHAPTER 16

SHORT- AND LONG-TERM N, P AND K REQUIREMENTS OF CASSAVA

Reinhardt Howeler[1]

INTRODUCTION

Throughout the tropics and subtropics cassava is grown on a wide range of soils, the main limitation being that the soils have to be reasonably well drained. **Table 1** shows that in Latin America most cassava is grown on Ultisols, Alfisols and Oxisols, while in Asia by far most cassava is grown on Ultisols, followed by Inceptisols, Alfisols and Entisols. In contrast to Latin America, in Asia very little cassava is grown on Oxisols and at elevations above 1000 masl. Except for the Alfisols, most cassava soils are characterized by a low pH, and low levels of N, P and K. Cassava can grow well on Mollisols and the better-drained Vertisols, but these highly fertile soils are generally used for higher-value crops such as sugarcane, maize, sorghum, soybeans and cotton.

Table 1. Soils on which cassava is produced in Latin America and Asia, and their principal nutritional constraints for the crop.

	Cassava production (%)		Constraints			
Soil Order	Latin America [1)]	Asia [2)]	Acidity	N	P	K
Ultisols	27	55	+	+	+	++
Alfisols	23	11	-	-	-	-
Oxisols	19	<1	++	+	++	++
Entisols	13	9	-	++	+	++
Inceptisols	7	18	++	+	++	+
Mollisols	6	2	-	-	-	-
Vertisols	4	3	-	-	-	-
Aridisols	<1	<1	-	-	-	-
Histosols	<1	<1	++	-	-	+

[1)] **Source**: Agro-ecological Studies Unit, CIAT, 1985.
[2)] **Source**: Howeler, 1992.

Even though cassava performs better than most crops on acid and infertile soils, the crop is highly responsive to fertilizer applications. Still, fertilizers or lime are seldom applied to the crop since farmers generally belief that the crop does not need good fertility and does not respond to fertilizers. However, thousands of fertilizer experiments conducted by FAO throughout the world between 1961 and 1977 (FAO, 1980) indicate that cassava is as responsive to fertilizer applications as other crops that traditionally are fertilized, and that fertilizer application to cassava can be highly economic **(Table 2)**.

[1] Formerly, CIAT cassava agronomist and soil scientist at CIAT, Dept. of Agriculture, Chatuchak, Bangkok 10900, Thailand. Currently, cassava consultant. r.howeler@cgiar.org

Table 2. The average highest percent yield increase due to fertilization and value/cost ratio (VCR) for cassava as compared to other crops in various countries.

Country	Crop	No. of trials	Average best	
			% Response	VCR
Brazil	Cassava	66	111.6	5.63
1970-‘76	Maize	510	83.1	2.74
	Cotton	490	84.2	3.73
	Beans	391	91.1	4.89
	Rice	385	76.6	4.81
	Soybean	124	102.4	2.38
	Sugarcane	105	66.6	3.64
Colombia	Cassava	16	124.5	5.89
1962-‘70	Maize	102	95.2	6.38
	Beans	47	67.2	5.96
	Forage	41	153.6	1.68
	Potatoes	33	266.4	11.40
	Wheat	15	72.8	4.43
Ghana	Cassava	134	71.0	19.90
1961-‘75	Maize	775	121.2	9.59
	Groundnut	134	52.1	18.70
	Cotton	92	82.1	18.31
	Cowpeas	61	65.1	15.90
Nigeria	Cassava	28	53.5	11.26
1961-‘77	Maize	478	64.1	5.12
	Yams	348	43.5	22.60
	Rice	277	41.8	13.78
Indonesia	Cassava	56	176.4	4.19
1969-‘76	Rice	378	62.6	3.12
	Sorghum	312	217.0	2.46
	Groundnut	135	60.0	4.88
	Soybean	117	59.9	3.12

***Source**: FAO, 1980.*

However, cassava is quite sensitive to over-fertilization, especially with N, which will result in excessive leaf formation at the expense of root growth. Cock (1975) reported that cassava has an optimal leaf area index of 2.5-3.5 and that high rates of fertilization may lead to excessive leaf growth and a leaf area index of >4. High N applications not only reduce the harvest index (HI) and root yield, but can also reduce the starch and increase the HCN content of the roots. Moreover, nutrients generally interact with each other, and the excessive application of one nutrient may induce a deficiency of another. Howeler *et al*. (1977) and Edwards and Kang (1978) have shown that high rates of lime application may actually reduce yields by inducing Zn deficiency. Spear *et al*. (1978) showed that increasing the K concentration in nutrient solution decreased the absorption of Ca and

especially Mg, leading to Mg deficiency. However, in both nutrient solution and field experiments with varying rates of applications of K, Ca and Mg, Howeler (1985b) did not find a significant effect of increasing K on the Ca concentration in the leaves. The Mg concentration decreased slightly in the field, but increased in the nutrient solution experiment. However, increasing the Mg supply markedly decreased the concentrations of K and Ca. Similarly, Ngongi *et al*. (1977) reported that high applications of KCl induced S deficiency in a low-S soil in Colombia; while Nair *et al*. (1988) found that high rates of P application induced Zn deficiency. Hence, it is important not only to apply the right amount of each nutrient, but also the right balance among the various nutrients.

Short- *vs* Long-term Responses to Fertilization

Short-term fertilizer experiments are usually conducted for 1-2 years at any particular site, while long-term experiments may be conducted for many years at the same site, applying the same fertilizer treatments to the same plots in every consecutive crop cycle. The short-term responses to the various applied nutrients depend largely on the original fertility characteristics of the soil as well as on the nutrient requirements of the test crop. In long-term experiments the response to particular nutrients may change over time, depending initially on the original fertility of the soil, but subsequently this will more and more depend on which nutrients are being depleted most by the removal of the harvested products.

The fertilizer experiments conducted by FAO, shown in **Table 2,** are mostly short-term trials. These indicate that in West Africa (Ghana) cassava responded mainly to K, in Latin America (Brazil) to P; and in Asia (Indonesia) to N, followed by K and P (Richards, 1979).

In nearly 100 NPK cassava trials conducted in Thailand in the early 1980s, the crop also responded mainly to N, followed by K and P (Hagens and Sittibusaya, 1990).

In 39 short-term NPK trials conducted in 9 states of Brazil from 1950 to 1983, the main limiting nutrient was P in 25 trials, K in nine and N in only six trials (Howeler, 2002). Similarly, in 22 short-term NPK trials conducted from 1980 to 1982 in four zones of Colombia, there was a significant response to the application of P in 12 locations, to K in six locations and to N in only four locations (Howeler and Cadavid, 1990). It was found that cassava responded mainly to P applications in the low-P soils of the Eastern Plains and of Cauca Department, to K applications only in the Eastern Plains, and to N applications principally in the sandy, low-OM soils of the Atlantic Coast.

In Africa, significant responses to K have been found on strongly acid soils of eastern Nigeria (Okeke, unpublished) and on slightly acid soils (0.23 me K/100 g) of southwestern Nigeria (Kang and Okeke, 1984). Obigbesan (1977a) did not observe a significant K response on three soils of western Nigeria, nor did Takyi (1972) in Ghana. In Madagascar, however, Roche *et al*. (1957) and Cours *et al*. (1961) found that K was the main limiting nutrient, and applications of 110 kg K_2O/ha were recommended (Anon., 1952; 1953).

In a long-term NPK experiment conducted in Quilichao, Colombia, there was a highly significant response to the application of P and K in the first year, but not to N. But in the eighth year of continuous cropping there was no significant response to P, but a highly significant response to both N and K, and a significant interaction between N and K, and between P and K. In the absence of K there were no responses to either N or P. Potassium had clearly become the main limiting nutrient after several years of cassava production due to the large removal of K in each root harvest, which had resulted in a significant decrease in exchangeable K in the soil, from about 0.2 to 0.1 meq K/100 g (see Figure 5 in Chapter 15) (Howeler and Cadavid, 1990).

Similarly, many long-term NPK experiments conducted in Asia have shown that K deficiency usually becomes the main limiting factor when cassava is grown continuously on the same soil without adequate K fertilization. **Table 3** shows the response of cassava to annual applications of N, P and K after several years of continuous cropping in 11 long-term experiments, which were conducted in four countries in Asia from 1987 to 1997. During the last year of these trials there was a significant or highly significant response to N in 8 trials, to K in 7 trials and to P in only 4 trials. But in three of four experiments that were continued for 16-20 years the main response was to the application of K, while in only one trial, at CATAS in Hainan, China, the main response was to application of N, in a sandy clay loam soil with only 0.54% OM (see Table 8 in Chapter 15).

Table 3. Response of cassava to annual applications of N, P and K after several years of continuous cropping in long-term trials conducted at various locations in Asia.

Country	Location	Years of cropping	Response to		
			N	P	K
China	-Guangzhou	4	**[1)]	**	**
	-Nanning	8	**	**	NS
	-Danzhou	6	**	NS	*
Indonesia	-Umas Jaya	10	NS	NS	NS
	-Malang	8	**	NS	**
	-Lampung	6	**	*	**
	-Yogyakarta	4	NS	NS	NS
Philippines	-Leyte	6	NS	NS	NS
	Bohol	4	**	NS	**
Vietnam	-Thai Nguyen	8	**	**	**
	-Hung Loc	8	**	NS	**

NS = no significant response
* = significant response (P<0.05)
** = highly significant response (P<0.01)
***Source**: CIAT, 1998*.

Thus, while in short-term fertilizer experiments there are often no significant responses to the application of chemical fertilizers, or the response is mainly to N and P, when these trials are continued in the same plots for many years, the response to fertilizers tends to increase over time due to the depletion of soil nutrients in the harvested roots. This is particularly the case for K, which is removed in large quantities in the roots, and for N, which may be removed in large quantities if leaves and stems are also taken from the field. Thus, in most cases K becomes the most limiting nutrient after several years of continuous cassava production in the same fields.

Nitrogen

Nitrogen is a basic component of protein, chlorophyl, enzymes, hormones and vitamins. It is also a constituent of the cyanogenic glycosides, linamarin and lotaustralin, which produce hydrocyanic acid (HCN) when cells are damaged. HCN is the bitter, highly toxic component of cassava leaves, stems and roots, which must be eliminated by drying or cooking the roots before consumption.

Cassava plants suffering from N-deficiency may not show any visible deficiency symptoms, but are shorter and grow less vigorous than normal. In some varieties and under severe N-deficiency leaves are slightly lighter green in color, the chlorosis being rather uniform throughout the plant. In nutrient solution trials, Forno (1977) observed only slight N-deficiency symptoms in cassava, while sorghum, maize and cotton showed severe symptoms. However, the growth of cassava was markedly reduced. This corresponds with observations at CIAT (Lozano *et al.,* 1981) in which N-deficiency in cassava resulted mainly in reduced growth rather than deficiency symptoms. However, this may vary with the variety being used; some varieties show a clear and rather uniform chlorosis of all leaves, while in other varieties the leaves remain dark green, while plant growth is reduced.

Significant responses to N have been observed more frequently in Asia than in Latin America or Africa. In nearly 100 NPK trials conducted by FAO on farmers' fields in Thailand, there was mainly a response to N, followed by K and P (Hagens and Sittibusaya, 1990). Similar results were obtained in 69 trials conducted in Indonesia (FAO, 1980). In Africa relatively few fertilizer trials have been conducted with cassava, mainly because very few cassava farmers apply fertilizers. In West Africa the responses to N were probably the most frequent (Okogun *et al*., 1999). In Latin America responses to N were the least frequent, with significant responses reported in only 5 out of 41 trials conducted in Brazil (Gomes, 1998) and in 5 out of 22 trials conducted in Colombia (Howeler and Cadavid, 1990).

Severe N deficiency is usually observed in very sandy soils low in OM, but may also be found in high-OM but acid soils, mainly due to a low rate of N mineralization. For instance, in Quilichao, Colombia, there was a highly significant response to the application of N in a volcanic ash soil with 7.1% OM but having a pH of 4.3 (Howeler and Cadavid, 1990). Some of the most dramatic responses to N have been obtained on the sandy soils of Jaguaruna in Santa Catarina state of southern Brazil. **Figure 1** shows a nearly linear response of two varieties up to levels of 150 kg N/ha. In this location yields increased from 10 t/ha to 35 t/ha by N application in a soil with 89% sand and 0.7% organic matter (Moraes *et al*., 1981). For both varieties highest yields were obtained with a fractionated

application with 1/3 applied at 30, 60 and 90 days after planting. Similar results were obtained in Carimagua, Colombia, where cassava responded to the application of 100 kg N/ha, with highest yields obtained with a fractionated application of 1/3 at 30, 120 and 150 days. However, the yield differences due to time of application were not statistically significant **(Figure 2)**. Trials on optimum time and fractionation of N applications have generally shown non-significant differences between single applications at planting, at one month after planting (MAP) or various fractionations (0-3 MAP) using N rates up to 100 kg N/ha (Howeler, 1985a). At higher rates, fractionation was found to be better than a single application.

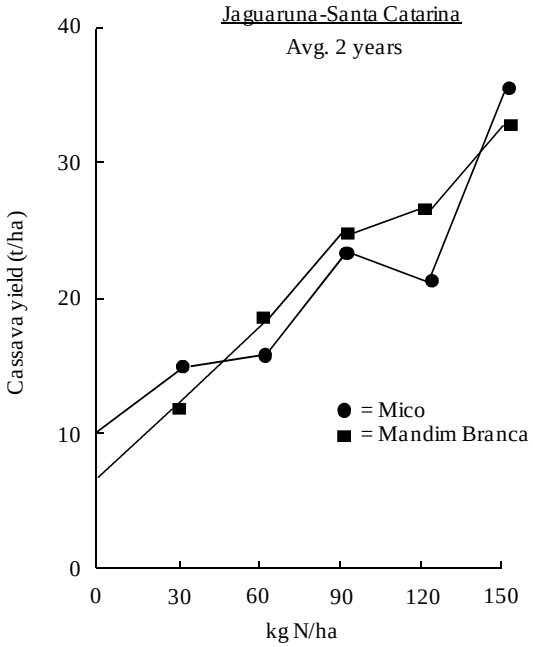

Figure 1. Response of two cassava varieties to different levels of application of N in a sandy soil of Jaguaruna, St. Catarina, Brazil.
***Source**: Moraes et al., 1981.*

A similar spectacular response to N was also observed in a clay soil with 1.2% OM in Jatikerto, East Java, Indonesia **(Figure 3)**. In this case, cassava was intercropped with maize, which competed strongly for the limited supply of N in the soil (Wargiono *et al*., 1998). In Kerala state of southern India cassava responds principally to the application of N, 100 kg N/ha being the recommended rate, half applied at planting and half at two months (Mandal *et al*., 1971). Similarly, in Thailand, where cassava is generally grown on moderately acid and low OM soils, the crop responds mainly to application of 50-100 kg N/ha (Sittibusaya *et al*., 1974).

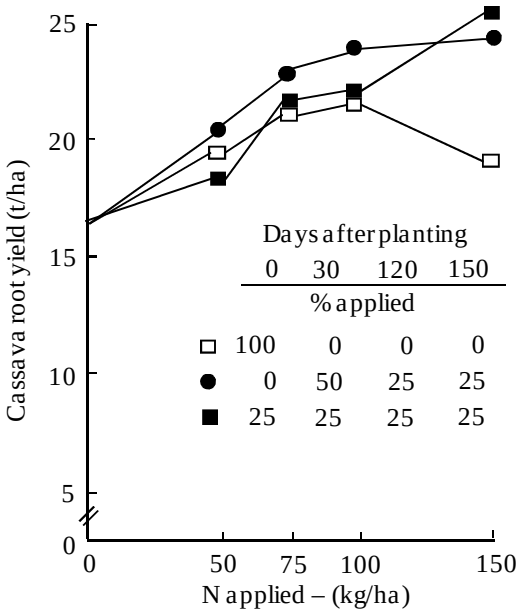

Figure 2. Response of cassava, cv. Llanera, to different levels and times of application of N in Carimagua, in the Eastern Plains of Colombia.

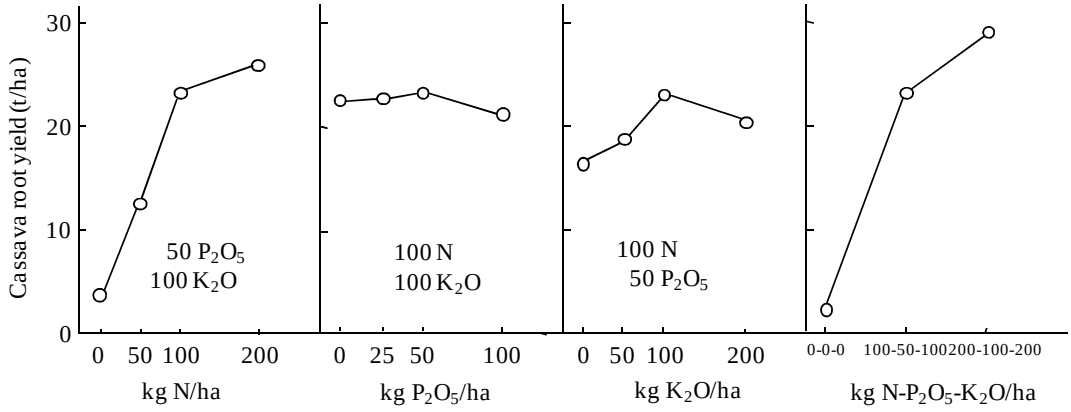

Figure 3. Response of cassava, cv. Faroka, to the annual application of various levels of N, P and K during the 7th crop cycle in Jatiderto, East Java, Indonesia in 1994/95.
Source: *Wargiono et al., 1998.*

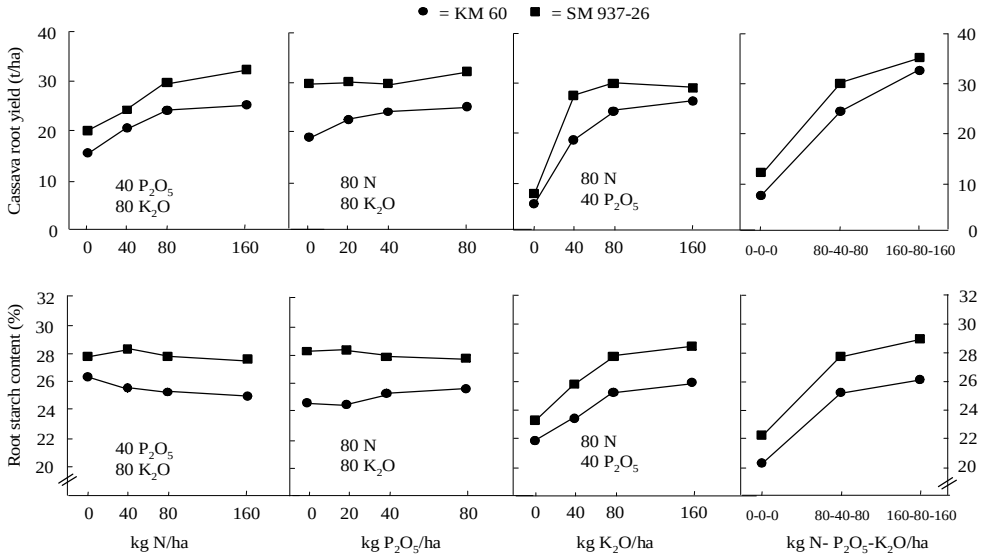

Figure 4. Effect of annual applications of various levels of N, P and K on the root yield and starch content of two cassava varieties grown at Hung Loc Agriculture Research Center in Trang Bon district, Dong Nai province, Vietnam in 2008/09 (19th year).

In Nanning, Guangxi, China, there was also a highly significant response to N, up to 200 kg N/ha in one cultivar (SC205), but only up to 50 kg N/ha in the other (SC201) (Zhang Weite *et al.*, 1998). As the latter cultivar is extremely vigorous, high N levels produced too much top growth at the expense of root production. Many investigators (Vijayan and Aiyer, 1969; Acosta and Perez, 1954; Obigbesan and Fayemi, 1976; Fox *et al.*, 1975) found that cassava responded negatively to high levels of applied N. This stimulates top growth excessively resulting in a reduction in root production. Krochmal and Samuels (1970) reported a root yield reduction of 41% and a top growth increase of 11% due to high N applications. Also, high levels of N application stimulate production of N-containing compounds such as protein and HCN, and may results in a decrease in starch content **(Figure 4).** High rates of N application may also increase the intensity of diseases such as cassava bacterial blight (Kang and Okeke, 1984). Thus, N rates must not only be adjusted to a particular soil but also tailored to the needs of a particular cultivar.

There are usually no significant differences among N sources such as urea, NH_4NO_3, and mono- or di-ammonium phosphate. Vinod and Nair (1992) reported significantly higher yields with slow-release N sources such as neem cake-coated urea or super-granules of urea.

High levels of N applications may be necessary for cassava forage production since the frequent cutting of tops will remove large amounts of N. **Figure 5** shows the response to N, P and K application in Carimagua, Colombia, both in terms of total dry forage and protein production as well as root yields. There was a highly significant response to application of all three nutrients up to the highest level of 200 kg/ha of N, P and K. Application of 200 kg N/ha increased total forage production from 3.3 to 6.3 t/ha and

protein yields from 0.7 to 1.4 t/ha. The latter corresponds to an N extraction of 224 kg/ha in the tops. The periodic cutting of tops affected cassava root yields and the response to fertilizers. Without N application, forage harvesting reduced root yields about 50%, while with 200 kg N/ha applied root yields decreased from 25 to 16 t/ha, corresponding to a 35% yield reduction. Application of the highest fertilizer level of 200 kg/ha of N, P and K resulted in the highest dry forage production of over 8 t/ha, equivalent to 2 t/ha of protein, while still producing 20 t/ha of fresh roots (CIAT, 1988a).

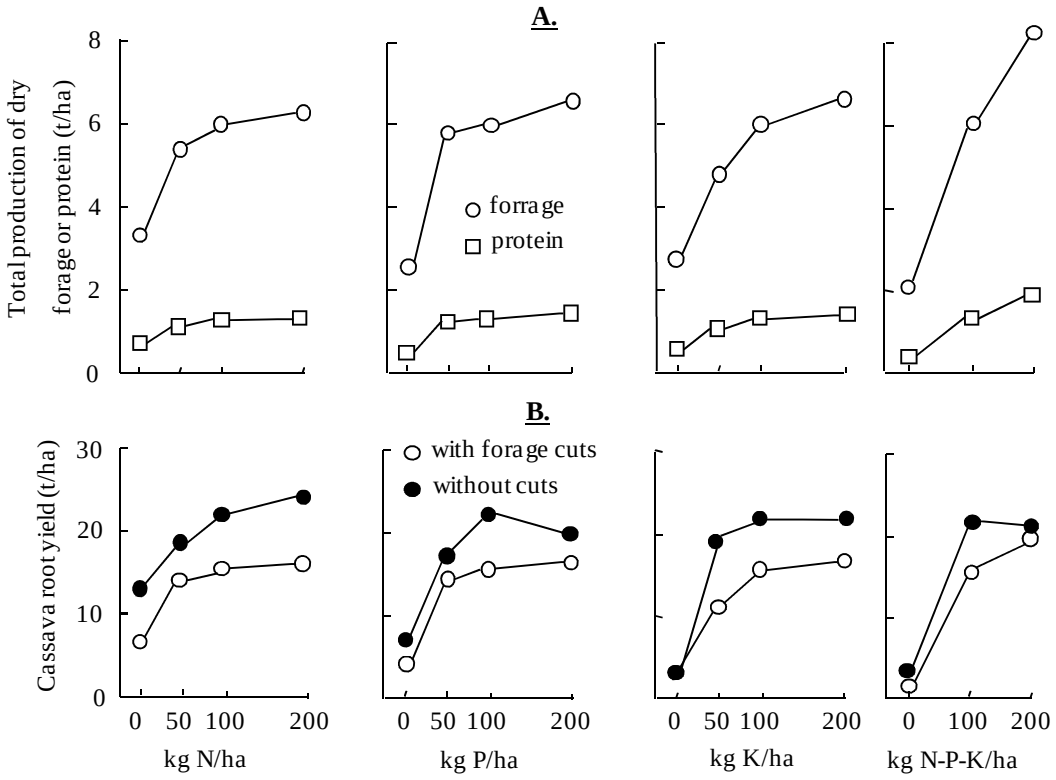

Figure 5. Effect of N, P and K application on total production of cassava dry forage and protein (A), as well as its effect on root production with or without forage cuts (B) of variety CM 523-7 during a 14 month crop cycle in Carimagua, Colombia. ***Source****: Howeler, 1985a.*

Similar results were obtained in Thailand by Putthacharoen *et al*. (1998), who reported a total N removal in roots and forage of 330 kg/ha during a 22-month crop cycle when green tops were cut at 3-4 month intervals. Thus, when cassava tops are cut off regularly for forage production, high rates of N (>200 kg/ha) need to be applied to sustain high levels of both shoot and root production.

Phosphorus

Phosphorus is a basic component of nucleoproteins, nucleic acids and phospholipids as well as all enzymes that play a role in energy transfer. Phosphorus is an important element for the process of phosphorilization, photosynthesis, respiration and the synthesis of carbohydrates, proteins and fats. Through these processes an adequate P supply is essential for the synthesis of starch and thus for normal root production. Malavolta *et al.* (1952) reported a reduction from 32% to 25% of starch in cassava roots when P was not supplied in a nutrient solution experiment, while Muthuswamy *et al.*(1974) reported no effect of P on the HCN content of roots

Roots contain relatively small amounts of P, and P removal from the soil in the root harvest is therefore much lower than that of N or K. However, in Latin America, where the majority of the cassava growing areas are characterized by extremely P-deficient soils, this element most limits cassava yields, at least in those fields where P fertilizers have not been applied before.

P deficient plants seldom show clear deficiency symptoms; instead, they are shorter and less vigorous, have thinner stems and smaller and narrower leaves than normal plants. Root yields can be seriously depressed by P-deficiency. Only in case of extreme deficiency, plants have a few dark yellow or orange lower leaves, which later become necrotic, flaccid and fall off. In the absence of clear deficiency symptoms, P-deficiency is generally diagnosed from the knowledge about the soil, or from soil or plant tissue analyses. When the soil contains less than 4-5 ppm Bray-II extractable P, or YFEL-blades have less than 0.4% P at 3-4 months of age of the plant, it is very likely that the crop will respond to P application.

Cassava's tolerance to low P concentrations in soil solution is not due to the efficient uptake of P by the root system; in fact, cassava grown in flowing nutrient solution required a much higher P concentration for maximum growth than rice, maize, cowpeas or common beans (Jintakanon *et al*., 1982; Howeler *et al*., 1981; Howeler 1990). When inoculated with endotrophic vesicular arbuscular mycorrhiza (VAM), the growth of cassava in nutrient solution improved significantly (Howeler *et al*., 1982a). Masses of mycorrhizal hyphae growing in and around the fibrous roots of cassava markedly increased the plant's ability to absorb P from the surrounding medium **(Photo 1)**. When planted in natural soil, the crop's fibrous roots soon become infected with native soil mycorrhizae. The resulting hyphae grow into the surrounding soil and help in the uptake and transport of P to the cassava roots. Through this highly effective symbiosis, cassava is able to absorb P from soils with low levels of available P, mainly by extending the soil volume from which P can be absorbed through the associated mycorrhizal hyphae (see Chapter 19).

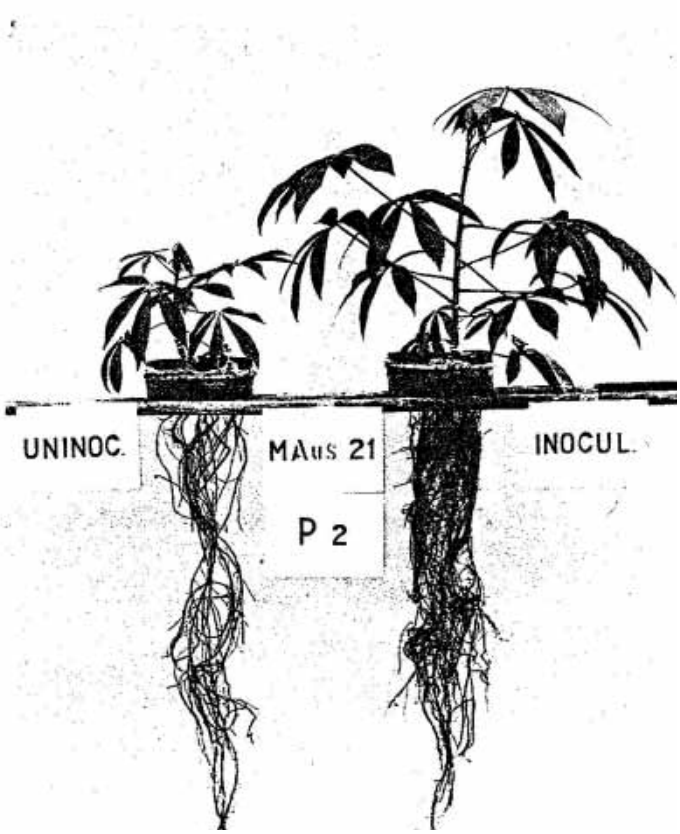

Photo 1. Cassava, cv. MAus 21, grown in flowing solution culture with 1 µM phosphate, with (right) and without (left) mycorrhizal inoculation

It has been clearly shown (Yost and Fox, 1979; van der Zaag *et al.*, 1979; Howeler *et al*., 1982a) that cassava is extremely dependent on an effective VA-mycorrhizal (VAM) association for absorption of P from the soil. In soils with a low or ineffective native mycorrhizal population cassava growth and production can be greatly increased by soil inoculation with a highly effective strain of mycorrhiza (see Chapter 19). In the presence of an effective mycorrhizal population, cassava is extremely tolerant of low levels of available P. While maize and soybean have a critical soil P level of 14-15 ppm, cassava requires only 8 ppm Bray-I extractable P (Kang *et al.*, 1980). **Table 4** shows that in nutrient solutions in the absence of a mycorrhizal association cassava has a very high P-requirement due to a coarse and inefficient root system. However, in natural soils in the presence of an effective VAM population cassava is extremely efficient in P-uptake and has a low external P requirement.

Table 4. External P requirement of various crops in terms of "available" soil P concentration in soil or nutrient solution (data are in ppm)

Crop	Soil-extract	Soil solution	Nutrient solution
Cassava	8 (Bray I) [1)]	0.01-0.04 [3)]	0.9-2.4 [4)5)6)]
	6 (Bray II) [2)]		
Maize	14 (Bray I) [1)]	0.06 [7)]	0.03 [5)]
Phaseolus beans	18 (North Carolina) [8)]	0.06 [9)]	0.03 [6)]
	10-15 (Bray II) [10)]		
Cowpea		0.016-0.1 [11)]	0.03 [6)]
Soybean	15 (Bray I) [1)]	0.018-0.2 [11)]	0.02 [5)]
Rice		0.03-0.12 [12)]	
Sorghum		0.05 [7)]	
Sweet potato		0.10 [3)7)]	
Irish potato		0.20 [3)7)]	
Chinese cabbage		0.20 [7)]	
Lettuce		0.40 [7)]	
Cotton			0.02 [5)]

References:
[1)] Kang *et al.*,1980.
[2)] CIAT, 1985.
[3)] van der Zaag *et al.*,1979.
[4)] Asher and Edwards, 1978.
[5)] Jintakanon *et al*., 1982.
[6)] Howeler *et al*. 1982a.
[7)] Fox *et al*., 1974.
[8)] Goepfert, 1972.
[9)] CIAT, 1978.
[10)] Howeler and Medina, 1978.
[11)] IITA, 1981
[12)] IITA, 1982

Severe P deficiency has been reported mainly in Latin America, particularly on Oxisols, Ultisols and Inceptisols in Brazil and Colombia. These soils are highly P-fixing and have available (Bray II or Mehlich I) P levels of only 1-2 ppm. During the first year(s) of cropping, cassava responds markedly to P application; but with continuous cropping on the same land, responses to P become less significant as soil P levels build up from previous applications (Nair *et al.*, 1988; Howeler and Cadavid, 1990; Kabeerathumma *et al.*, 1990).

In Asia, P deficiency is seldom the principal limiting factor for cassava production because most cassava is grown on soils with more than 4 ppm of available P, or on soils that had previously been fertilized with P. Nevertheless, significant responses to P application have been observed in Guangzhou (Guangdong), in Nanning (Guangxi) and on Hainan Island of China; in northern and southern Vietnam; and on Leyte Island of the Philippines. In low-P soils in Kerala State, India, significant initial responses to 100 kg P_2O_5/ha were reported; but these declined over time. Nair *et al*. (1988) determined an optimum economic rate of 45 kg P_2O_5 /ha. The most marked responses to P application in Asia were observed in the Plain of Jars of northeast Laos in soils with only 0.9 ppm Bray-II extractable P **(Figure 6)** (CIAT, 2007).

In Africa few P experiments have been conducted with cassava. Responses to P application have been reported mainly in Ghana (Stephens, 1960; Takyi, 1972) and Madagascar (Cours *et al*., 1961). Ofori (1973) reported a negative effect of P application on cassava yields on a forest Ochrosol in Ghana.

Large varietal differences have been observed in cassava's ability to grow on low-P soils (CIAT, 1988a, b). Pellet and El-Sharkawy (1993a, b) found that varietal differences in response to applied P were not due to genetic differences in P-uptake efficiencies, but rather to contrasting patterns of DM distribution and P-use efficiency (root yield/total P in plant). Low-P tolerant cultivars had a high fine root-length density, moderate top growth, and a high and stable harvest index (HI).

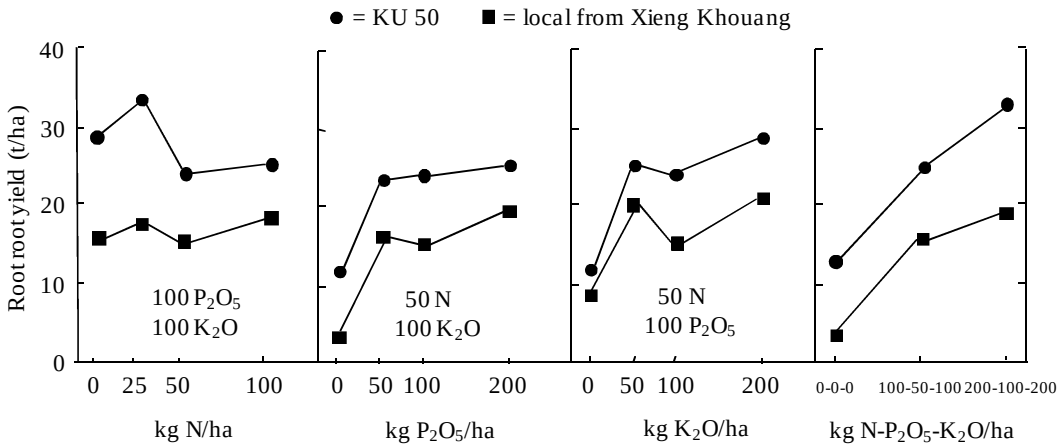

Figure 6. Effect of the application of various levels of N, P and K on the root yield of two cassava varieties grown at the Cattle Bank in Paek district, Xieng Khouang province of Lao PDR in 2005/07 (two year crop).
***Source**: CIAT, 2007.*

Responses to P application depend on the available-P level of the soil, the mycorrhizal population and the variety used. Van der Zaag *et al*. (1979) reported high yields of 42 t/ha in an Oxisol in Hawaii with only 3 ppm P ($NaHCO_3$-extractant) using the cultivar Ceiba. CIAT (1988a) similarly reported that some varieties produced yields of 40-50 t/ha without P application in a soil with only 4.6 ppm P (Bray II). In other soils with equally low levels of available P but with a less-efficient mycorrhizal population, cassava responded very markedly to P applications. Thus, in the Oxisols of the Eastern Plains of Colombia, with only 1.0 ppm P (Bray II), cassava responded markedly to applications of 200-400 kg P_2O_5/ha **(Figure 7)**. Of the seven P sources tested, banding of triple superphosphate (TSP) or broadcast applications of basic slag were most effective. Partially acidulated rock phosphate or rock phosphate mixed with elemental sulfur (S) were also quite effective in these acid soils (CIAT, 1978). Locally produced simple superphosphate (SSP) was less effective, except at high rates of application. Similarly, Santos and Tupinamba (1981) reported significant responses to 60 or 120 kg P_2O_5 /ha in three soils of Sergipe, Brazil, with TSP and hyperphosphate being more effective than two local sources of rock phosphate. Soluble-P sources like TSP, SSP, mono- or di-ammonium phosphate,

should be band applied near the stakes; while less-soluble sources such as basic slag and rock phosphates should be broadcast and incorporated. All P should be applied at or shortly after planting as fractionation of P had no significant effect on yield. Alternative methods of P application, such as stake treatments or foliar sprays, are not as effective as soil application in increasing yields (Howeler, 1985a).

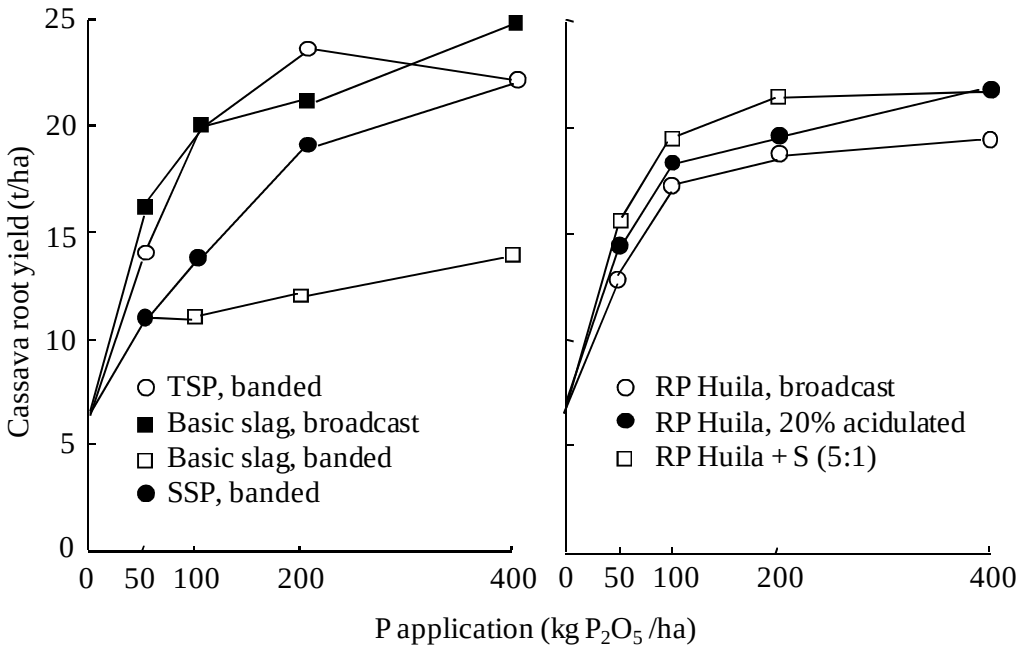

Figure 7. The effect of different levels, sources and methods of application of P on the root yield of cassava, cv. Llanera, grown in Carimagua, Meta, Colombia.
***Source**: Howeler, 1985a.*

Potassium

Potassium is not a basic component of protein, carbohydrates or fats, but plays an important role in their metabolism. Potassium stimulates net photosynthetic activity of a given leaf area and increases the translocation of photosynthates to the tuberous roots. This results in low carbohydrate levels in the leaves, further increasing photosynthetic activity (Kasele, 1980).

Blin (1905), Obigbesan (1973) and Howeler (1998) reported that K application not only increased root yields but also their starch content. Similar increases in starch content with increasing applications of K have been observed in Carimagua (CIAT, 1982a) and Pescador, Colombia (Howeler, 1985a), as well as in southern Vietnam (Nguyen Huu Hy *et al*., 1998) and China (Howeler, 1998). In general, root starch content increases up to 80-100 kg K_2O/ha and then levels off or decreases at higher rates of K application (see **Figure 4**). Obigbesan (1973) and Kabeerathumma *et al*. (1990) reported that K application also

decreased the HCN content of roots, while Payne and Webster (1956) found highest levels of HCN in roots produced in low-K soils.

Like N and P, deficiency of K results mainly in reduced plant height and vigor. Stem internodes are markedly reduced and the upper stem tends to lignify prematurely, resulting in a zigzag growth. In general, stems are thick and highly branched, producing a prostrate growth habit. Clear deficiency symptoms in leaves are seldom observed. In pot and nutrient solution experiments K-deficient plants have often small and light green leaves at the top of the plant. In the field K deficient plants are seldom chlorotic, but upper leaves are small and lower leaves may be yellow and necrotic along the borders. Some of this necrosis seems to be due to K-deficiency induced diseases, mainly anthracnose. The edges of lower leaves may also curl up, similar to drought symptoms.

Potassium deficiency in cassava is generally found in tropical soils with low-activity clay such as in Oxisols, Ultisols and Inceptisols, as well as in Alfisols derived from sandstone. After land clearing the Alfisols have a reasonable level of exchangeable K, but often show a significant K response in the second year of planting because of low K reserves in the parent material (Kang and Okeke, 1984). Most light-textured soils have low K reserves, which are rapidly depleted after one or more cassava harvests.

Long-term experiments in Asia and Colombia have shown that K deficiency almost invariably becomes the main limiting nutrient when cassava is grown continuously on the same soil without adequate K fertilization. **Figures 8** and **9** show the results of a long-term NPK trial conducted on a light-textured soil at Thai Nguyen University in Thai Nguyen, North Vietnam. Two cassava varieties were grown in the same plots with the same annual applications of N, P and K for 17 years. During the last year the average yield increased from 3.40 to 21.78 t/ha with the application of 80 kg K_2O/ha, but did not increase further with the higher rate of application of 160 kg K_2O/ha. **Figure 8** also shows that lack of adequate N and K drastically reduced leaf life, i.e. the average number of days between leaf formation and leaf fall, while lack of P did not have a similar effect. Thus, relatively high yields of about 20-25 t/ha could be maintained during 17 years of continuous cropping with the annual application of 80 kg of N, 40 P_2O_5 and 80 K_2O/ha. However, the exchangeable K content of the soil did not increase with these rates of K application and remained far below the critical level at around 0.06 meq/100 g **(Figure 9)**.

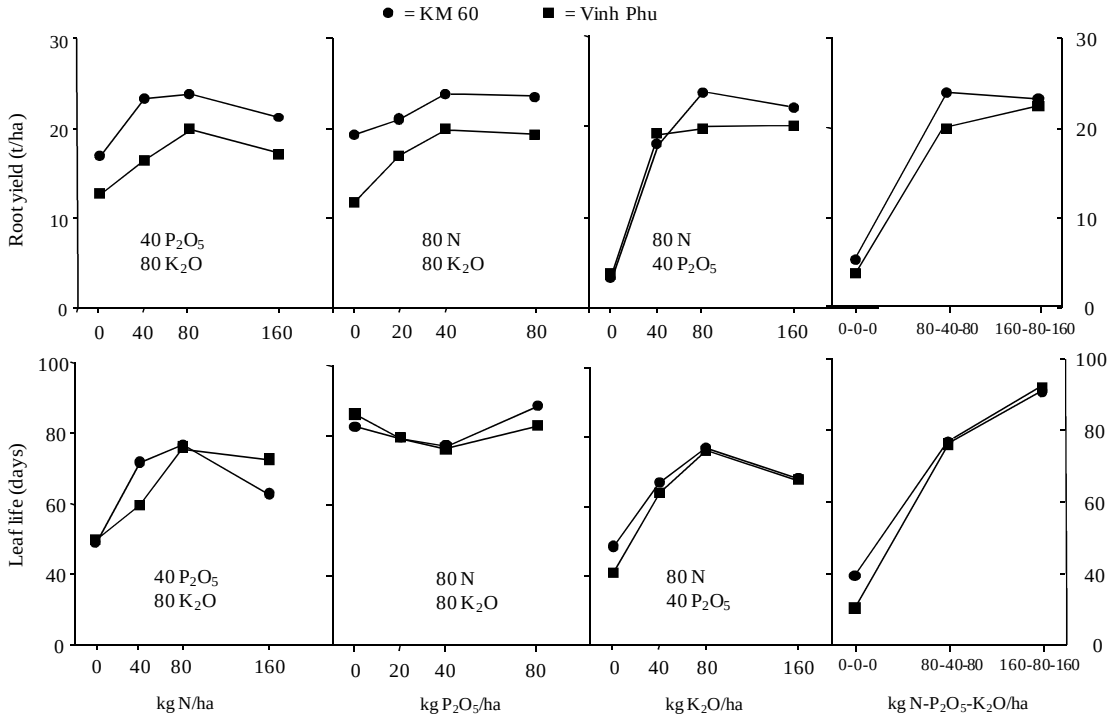

Figure 8. Effect of annual applications of various levels of N, P and K on the root yield at 10 MAP and the leaf life at 3 MAP of two cassava varieties during the 17th consecutive crop cycle at Thai Nguyen University in Thai Nguyen, North Vietnam in 2006.
***Source**: Tran Ngoc Ngoan, personal communication*

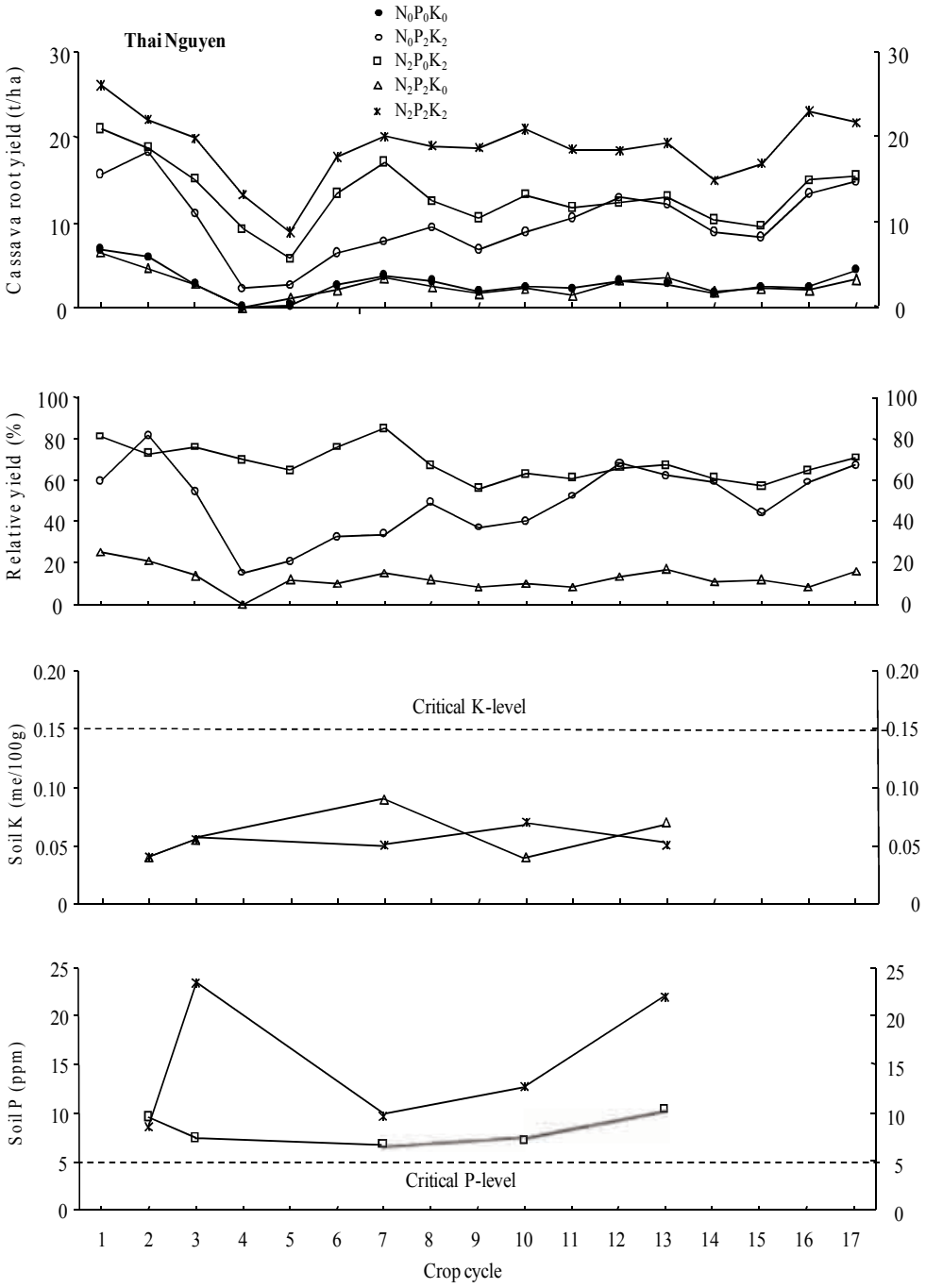

Figure 9. Effect of annual application of N, P and K on cassava root yield, relative yield (yield without the nutrient over the highest yield with the nutrient) and the exchangeable K and available P (Bray 2) content of the soil during 17 years of continuous cropping in Thai Nguyen University of Agriculture and Forestry, Thai Nguyen, Vietnam.

In a very poor sandy soil near the Atlantic Coast of Colombia, Cadavid *et al*. (1998) also found that annual applications of 50 kg N, 50 P_2O_5 and 50 K_2O/ha increased yields during eight years of continuous cropping, but had no effect on soil K, which remained at a low level (0.06 meq/100 g).

Figure 10 shows the response to K application during four years of consecutive cropping in Carimagua, Colombia, in a soil with only 0.08 meq exchangeable K/100 g. In the first year there was no response to K application, but in subsequent crops the response became more marked. In the fourth year the yield of the K check plot was only 7.8 t/ha compared with 20 t/ha at the highest rate of 200 kg K_2O/ha.

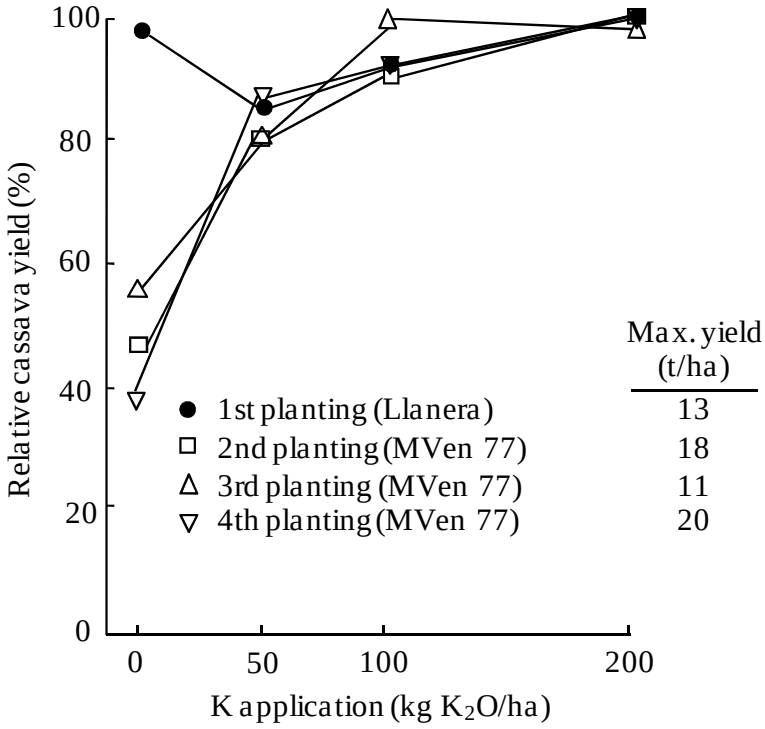

Figure 10. The effect of annual application of four levels of applied K on the relative yields of cassava, cv. MVen 77, during four consecutive cropping cycles in Carimagua, Colombia. ***Source****: Howeler, 1985a.*

Many experiments on time of application of K have given somewhat contradictory results. In India, Kumar *et al*. (1971) recommended the application of K half at planting and half side-dressed at 1 month, whereas Ashokan and Sreedharan (1977) recommended a split application only when small amounts of K are applied. In the same country, the Central Tuber Crops Research Institute (CTCRI, 1972) reported no significant differences between a full basal application or half basal application and half application at 2 months.

Similar results have been reported by CIAT (1977, 1978, 1982a, 1982b). A basal application at 30 days after planting produced the highest overall yields **(Figure 11)**. Thus, it appears that split applications of K are generally not necessary but may have some advantages on well-drained soils and with low rates of K application.

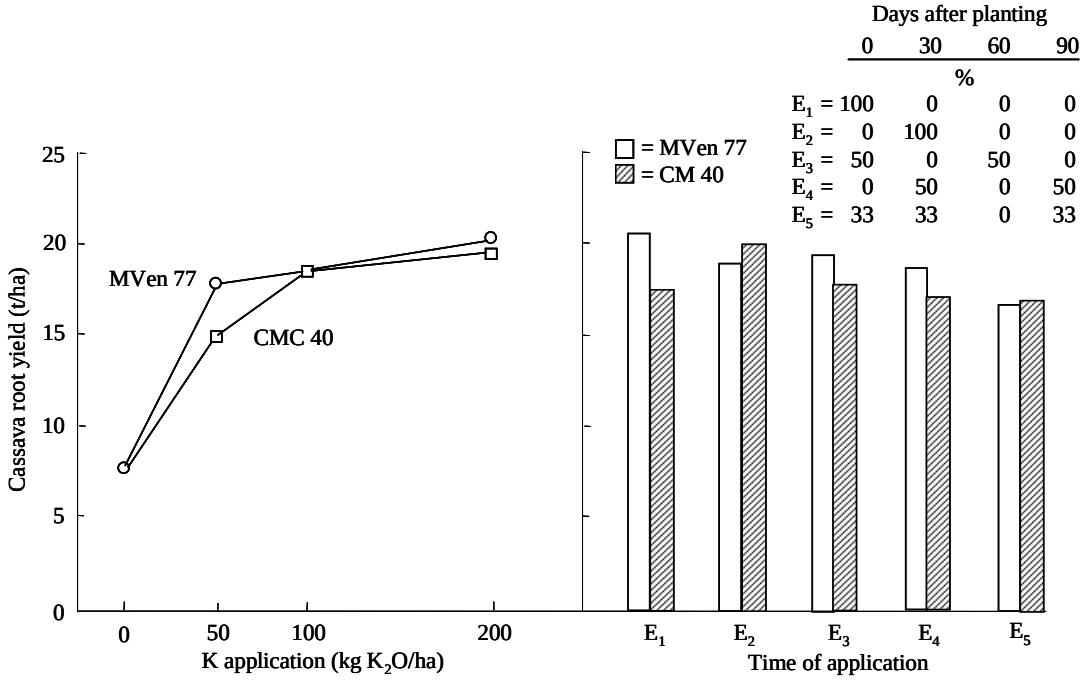

Figure 11. Effect of levels and times of application of K on the root yield of two cassava varieties grown in Carimagua, Meta province of Colombia.

Among different K sources, KCl is the cheapest and most commonly used source. Ngongi *et al.* (1977) showed that KCl and K_2SO_4 were equally effective K sources, except in soils with low S contents. In those it is recommended to use K_2SO_4 or mix elemental S with KCl to prevent the induction of S deficiency by high applications of chlorides.

Effect of Fertilizers on Root Quality

Fertilizer applications do not only affect cassava yields, but also the quality of the harvested roots, primarily the dry matter and starch contents of the roots as well as the HCN contents, and thus the bitterness of the roots. Chan and Lee (1982) reported that root starch contents increased with K application, reaching a maximum of 36.8% with the application of 180 kg K_2O/ha. Higher K rates decreased the starch content. Obigbesan (1973) also found a marked effect of K application on starch content, being maximum with 67 and 100 kg K_2O/ha applied, whereas the HCN content decreased from 270 to 160 ppm of fresh roots with the application of 134 kg K_2O/ha. However, in other trials, Obigbesan (1977b) found no effect of K on DM or starch contents and no significant effect on the HCN content of roots. The Central Tuber Crops Research Institute (CTCRI, 1975) reported a slight increase in starch content due to K application, but a marked decrease in

HCN content of the roots. CIAT (1980) also reported an increase in starch content from 26.7 to 34.2% with the application of 50 kg K_2O/ha, above which there was no significant effect. In NPK trials in Colombia, it was found that in most cases K application had no significant effect on starch content. Only in two out of 19 trials was there a significant positive effect, whereas in one trial the effect was negative. Thus, it appears that the effect of K on starch content is rather variable; in low K soils (<0.15 meq K/100g) there is generally a positive effect with low levels of application, above which there is not a significant effect.

High rates of N application, on the other hand, will generally decrease the root starch content (see Figure 6 in Chapter 15), while it will increase the production of N-containing compounds such as proteins and HCN. P application tends to increase the root starch content, but not to the same extent as the application of K. **Figure 12** shows that at a high elevation site in Cauca Department of Colombia, application of 100 kg K/ha increased the root starch content from 32% to 35%. Higher applications of K had no more beneficial effect. P application up to 100 kg P/ha (229 kg P_2O_5/ha) also increased the starch content, while N application had no effect at low levels of application and decreased starch content at rates of 200 kg N/ha.

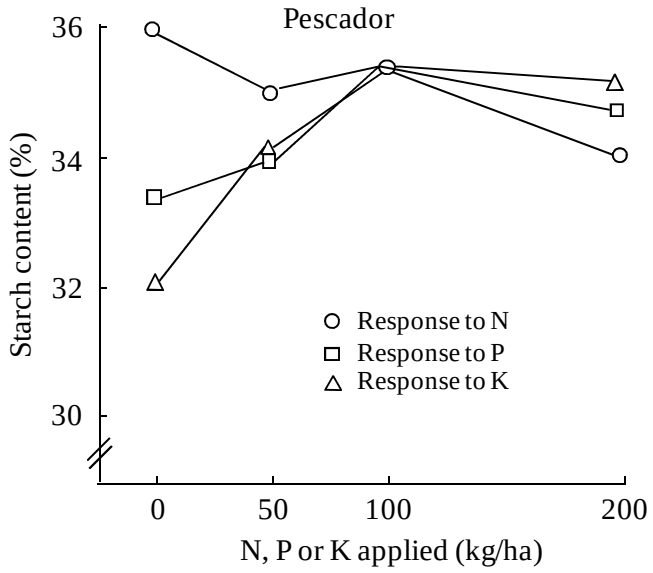

Figure 12. Effect of different levels of applied N, P and K on the starch content of cassava roots in Pescador, Cauca, Colombia.
***Source**: Howeler, 1985a.*

Effect of Fertilizers on Stake Quality

Fertilizer application does not only affect root yields and the quality of the roots, but it also affects top growth, the thickness of stems, and ultimately the quality of stakes produced from those stems, which in turn affect the yield of the following crop. **Table 5**

shows that when stakes were cut from plants that had been fertilized with different combinations of N, P and K during the previous eight years in Quilichao, Colombia, these stakes had markedly different nutrient contents, depending on the previous fertilization of the mother plants. This was partially due to the well-fertilized plants producing thicker stakes (dry weight per stake increased from 11.0 to 16.0 g/stake) and partially due to an increase in the concentration of each nutrient in the stake; this was especially true for K. Moreover, the previous fertilizer treatments increased the starch and sugar contents of the stakes, which are important for improving sprouting and the early vigor of the new plants. This was clearly evident in the significant improvement in sprouting, and ultimately resulted in marked improvements in top growth and root production of the following cassava crop. Fertilizer application of that crop did increase yields, but the previous fertilization of the mother plants had an even more marked affect on yields, almost doubling yields under both fertilized and unfertilized conditions of the crop (**Table 5**) (Lopez and El-Sharkawi, 1995). Similar results were obtained by Keating *et al*. (1982).

Table 5. Effect of N, P and K fertilization of mother plants on the quality of stakes cut from the stems, and on the sprouting and yield of the subsequent cassava crop, in CIAT-Quilichao, Colombia in 1991/92.

Treatments [1]			Nutrient content of stakes			Starch content of stakes	Sprouting of stakes	Unfertilized	Fertilized [2]
N	P	K	N	P	K			Root yield	
← (kg/ha) →			← (mg/stake) →			(g/stake)	(%)	← (t/ha) →	
0	0	0	70	10	19	2.62	85b	13.5	19.1
0	100	100	76	21	54	3.38	97a	17.5	24.6
100	0	100	146	14	87	4.68	98a	14.9	23.5
100	100	0	117	21	28	3.17	77b	15.8	24.7
100	100	100	139	25	72	4.29	97a	24.2	30.2

[1] Fertilization of mother plants from which stakes were cut

[2] Fertilization of subsequent crop with 50 kg N, 100 P_2O_5 and 100 K_2O

***Source**: adapted from Lopez and El-Sharkawi, 1995.*

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CHAPTER 17

SECONDARY AND MICRONUTRIENT REQUIREMENTS OF CASSAVA AND THE USE OF SOIL AMENDMENTS

Reinhardt Howeler[1]

Calcium and Magnesium

Calcium plays an important role in the supply and regulation of water in the plant, while Mg is a basic component of chlorophyll and as such is essential for photosynthesis.

Symptoms of Ca-deficiency are seldom observed on cassava in the field; but in very acid soils with low levels of exchangeable Ca (<0.25 meq/100 g), the crop may respond to Ca applications. Plants suffering from Ca-deficiency are slightly smaller and the fibrous root system is less developed. In nutrient solutions severe Ca-deficiency results in short plants, yellowing of leaf margins of older leaves and curling and puckering of leaf tips and margins of young leaves. Since Ca is a phloem immobile element, its deficiency affects principally the growing points of both tops and roots. Thus, Ca-deficiency reduces root growth and results in a coarse and stubby root system.

In flowing solution culture cassava was found to be more tolerant of extremely low levels of Ca than were maize, sorghum, sunflower and soybean (Edwards *et al*., 1977). Also, in very Ca-deficient soils in Nigeria, Edwards and Kang (1978) did not observe Ca deficiency symptoms in cassava, while maize, soybean and lima beans were severely affected.

In Carimagua-Alegría, Colombia, highly significant responses to application of Ca were obtained in a sandy loam soil with a pH of 5.1 and only 0.18 meq Ca/100 g and 0.05 meq Mg/100 g (**Figure 1**). Highest yields were obtained with application of 200-400 kg Ca/ha as broadcast gypsum. Broadcast calcitic or dolomitic limes were less effective, while band-applied gypsum was ineffective in increasing cassava yields (CIAT, 1985). As these Ca sources are relatively insoluble, they should all be broadcast and incorporated before planting. The good response to gypsum was not a response to S because either $MgSO_4$ or elemental S had been applied uniformly to all plots. Due to its low Ca content (8-11%) and high cost, gypsum is an expensive source of Ca compared with lime. However, **Figure 1** shows that 100 kg Ca/ha as gypsum was more effective than 400 kg Ca/ha as calcitic lime, both being equivalent to about one t/ha product to be applied.

Symptoms of magnesium deficiency are frequently observed in cassava grown on acid Oxisols, Ultisols, Inceptisols and Entisols. They are characterized by intervenal chlorosis and a distinct yellowing of the margins of lower leaves. Under very severe Mg-deficiency, plants are reduced in size and the lower leaves may be completely yellow with necrosis along leaf borders. Cassava was found to be quite susceptible to Mg deficiency, requiring for maximum growth higher Mg concentrations in nutrient solution than cowpea

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or cotton (Whitehead, 1979). Also, Mg deficiency symptoms were easily induced by high concentrations of K in nutrient solution (Spear *et al.*, 1978).

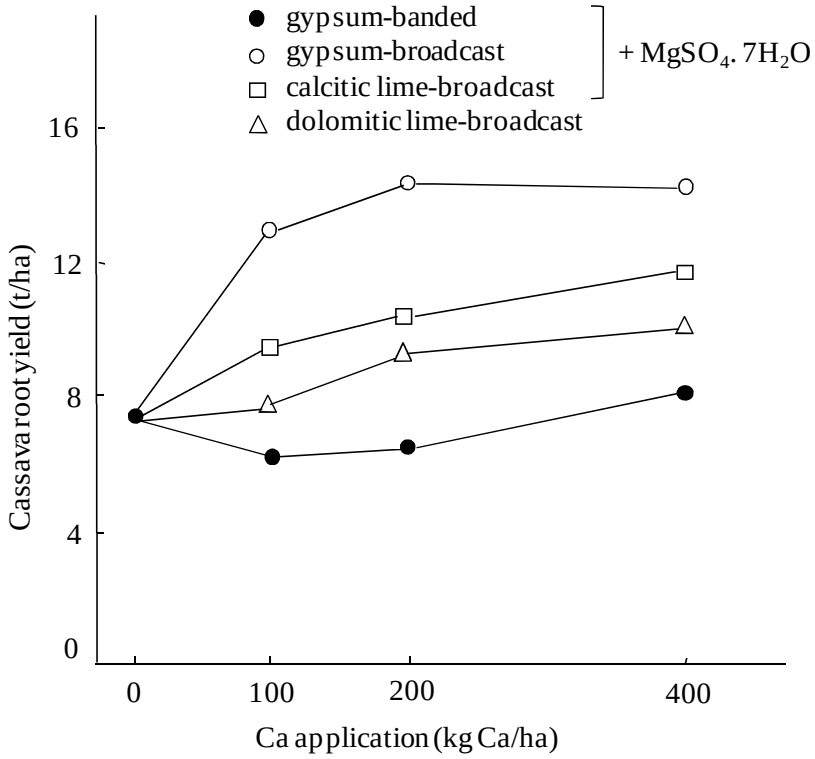

Figure 1. Effect of different levels, sources and methods of application of Ca on the fresh root yield of cassava, cv. CM 523-1, in Carimagua, Colombia. ***Source:*** *CIAT, 1985.*

In the same soil in Carimagua, two Mg-experiments were conducted to determine the optimum rates and best sources of Mg (CIAT, 1985). There was a significant response up to the highest level of 60 kg Mg/ha, but there were no overall significant differences among sources. The more soluble Sulphomag was more effective at intermediate rates, while banded $MgSO_4$ or broadcast MgO were better at higher rates of application (**Figures 2** and **3**).

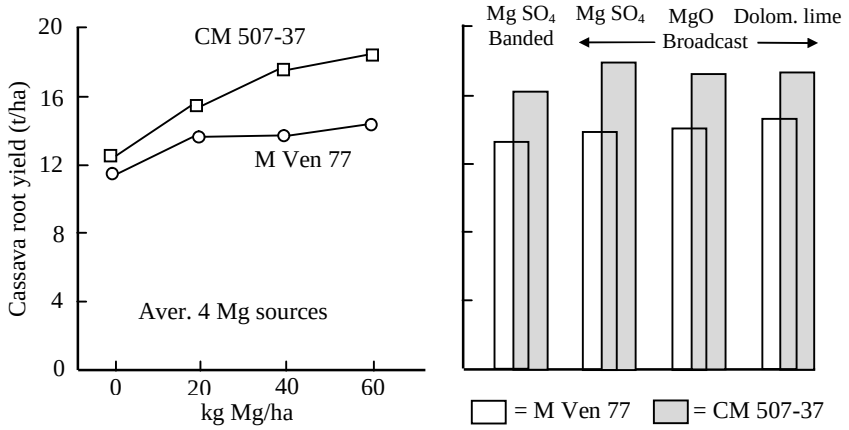

Figure 2. Response of two cassava varieties to different levels (left) and sources (right) of applied Mg in Carimagua, Colombia.
Source: *CIAT, 1985.*

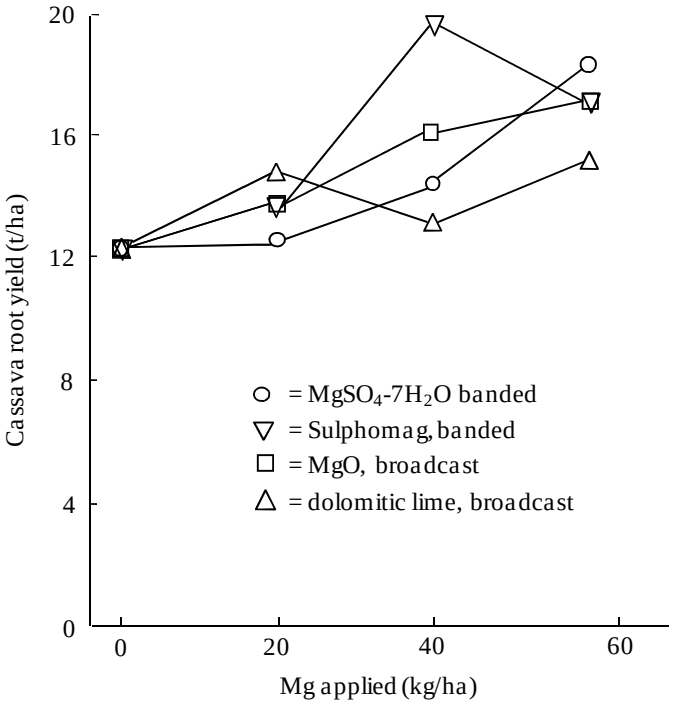

Figure 3. Response of cassava, cv. CM 430-37, to various levels of Mg applied as four different sources in Carimagua- Alegria, Colombia.

Interactions between Potassium, Calcium and Magnesium

There are numerous reports in the literature on the interaction between K, Ca and Mg in a range of crops, including such tropical crops as bananas (Lahav, 1974) and peanuts (Fageria, 1973). In general, it was found that increasing the application of K resulted in a decrease in the absorption of other cations, such as Ca and Mg. In the case of cassava, Spear *et al.* (1978) reported that in flowing solution culture in which the concentrations of K, Ca and Mg were closely controlled, increasing the concentration of K in solution from 0.5 to 8,024 µM resulted in an increase in K and a decrease in Ca and Mg absorption as well as the concentrations in plant tissue. In some cultivars the rate of Ca absorption increased with increasing K concentration between 0.5 and 6 µM but decreased at higher concentrations. The rate of Mg absorption was strongly depressed by increasing levels of K, and this resulted in the induction of Mg deficiency symptoms at high levels of applied K. Cassava had a lower rate of Mg absorption and a greater retention of Mg in the roots than maize and sunflower, making it inherently more susceptible to K-induced Mg deficiency. Conversely, cassava had a higher rate of Ca absorption than maize, and this rate was less affected by increasing the K concentration, making it less susceptible to K-induced Ca deficiency.

However, in nutrient solution studies in which K, Ca and Mg concentrations increased according to the rate of plant growth (programmed nutrient solution techniques, as described by Asher and Cowie, 1970), there was no consistent effect of increasing solution K concentrations on the Ca concentration of youngest fully expanded leaf (YFEL) blades, whereas that of Mg slightly increased (**Table 1**). Thus, when the Ca and Mg supply was sufficiently high to maintain an optimum rate of absorption throughout the 2-month growth period, there was no effect of K on Ca and Mg uptake. Conversely, when the solution Mg concentration increased, the K concentration of YFEL blades decreased markedly from 2.74 to 1.59%, while the Ca concentration decreased from 0.75 to 0.32%. With increasing Ca concentration in solution, there was a marked decrease in the Mg concentration of YFEL blades but no consistent effect on the K concentration (**Table 1**). Thus, under these experimental conditions, increasing the K supply had no effect on Ca and Mg concentrations, but increasing the Mg supply had a marked depressing effect on the K and Ca concentration in YFEL blades.

Field experiments with the same cultivar in Carimagua, Colombia (**Table 2**) showed that increasing applications of K slightly reduced the Mg concentration and had no effect on the Ca concentration in YFEL blades. Increasing applications of Ca had no significant effect on K but slightly depressed the Mg concentration, whereas increasing applications of Mg slightly decreased the concentrations of both K and Ca in YFEL blades.

The discrepancy in results between these three sets of trials is due mainly to the greater range of K, Ca and Mg concentrations used in the nutrient solution studies than in the field trials. If only the intermediate levels 3, 4, 5 and 6 in **Table 1** are compared with those of **Table 2**, one would find more correspondence of results. One could conclude that under normal field conditions, the application of K is not likely to have a significant effect on Ca, but may depress the Mg concentration in YFEL blades, whereas the application of increasing levels of Ca or Mg does not affect the concentration of K, but may depress the concentration of Mg and Ca, respectively, in the YFEL blades.

Table 1. Concentration of K, Ca and Mg in youngest fully expanded leaf blades of 2-month old cassava, cv. MVen 77, grown with increasing concentrations of each element in nutrient solution experiments at CIAT, Colombia.

K experiment				Ca experiment				Mg experiment			
K level applied	K (%)	Ca (%)	Mg (%)	Ca level applied	Ca (%)	K (%)	Mg (%)	Mg level applied	Mg (%)	K (%)	Ca (%)
K-1	0.85	0.37	0.25	Ca-1	0.05	1.95	0.31	Mg-1	0.05	2.74	0.75
K-2	1.43	0.40	0.24	Ca-2	0.11	1.70	0.27	Mg-2	0.07	2.27	0.67
K-3	1.16	0.35	0.28	Ca-3	0.33	1.81	0.26	Mg-3	0.15	1.68	0.43
K-4	1.35	0.42	0.27	Ca-4	0.47	1.65	0.22	Mg-4	0.20	1.67	0.41
K-5	1.68	0.51	0.29	Ca-5	0.52	1.73	0.21	Mg-5	0.22	1.69	0.37
K-6	1.90	0.39	0.28	Ca-6	0.57	1.87	0.18	Mg-6	0.24	1.54	0.35
K-7	2.36	0.32	0.29	Ca-7	0.72	1.76	0.16	Mg-7	0.30	1.59	0.32

***Source**: Howeler, 1985.*

Table 2. Effect of application of various levels of K, Ca and Mg on the concentration of these nutrients in youngest fully expanded leaf blades of 2-4 month old cassava, cv. MVen 77, grown in field experiments in Carimagua-Alegria, Colombia.

K experiment				Ca experiment				Mg experiment			
K applied (kg/ha)	K (%)	Ca (%)	Mg (%)	Ca applied (kg/ha)	Ca (%)	K (%)	Mg (%)	Mg applied (kg/ha)	Mg (%)	K (%)	Ca (%)
K-0	1.25	0.67	0.26	Ca-0	0.32	1.82	0.28	Mg-0	0.20	1.99	0.70
K-50	1.82	0.68	0.24	Ca-100	0.51	2.00	0.27	Mg-20	0.23	1.91	0.69
K-100	1.87	0.66	0.23	Ca-200	0.48	1.87	0.25	Mg-40	0.25	1.93	0.69
K-200	2.07	0.66	0.23	Ca-400	0.51	1.90	0.24	Mg-60	0.25	1.94	0.60

***Source**: Howeler, 1985.*

Sulfur

Sulfur is a basic component of several amino acids and therefore plays an important role in protein synthesis. When the S-supply is deficient the plant accumulates in its leaves excessive amounts of inorganic N, amino acids and amids, without sufficient protein production (Stewart and Porter, 1969).

Sulfur deficiency in cassava is characterized by a uniform yellowing of upper leaves similar to those caused by N deficiency. Usually, the whole plant becomes uniformly chlorotic and leaves remain small. This deficiency can be induced by high applications of KCl, and eliminated by applications of K_2SO_4 or other sulfate sources, as well as by incorporation of elemental S (Ngongi *et al*., 1977).

In industrial areas much of the plant's S requirements are met from S emissions into the atmosphere, but in isolated areas cassava may suffer from S deficiency. This has

been reported only for Carimagua in the Llanos Orientales of Colombia, which is far removed from any industrial center. Soils there contained only 23 ppm of Ca phosphate-extractable S; with application of 40 kg S/ha as elemental S this increased to 36 ppm. **Figure 4** shows a clear response to the application of S up to 20-40 kg S/ha. There were no significant differences among S-sources although yields were slightly higher with banded K- and Mg-sulfate than with broadcast elemental S. Clear S-deficiency symptoms were observed in the check plots. These plants had 0.20-0.25% S in YFEL blades, compared with 0.30-0.32 % in plants that had received S applications. Critical levels of 0.27 and 0.33 % S were estimated in two field experiments (Howeler, 2002).

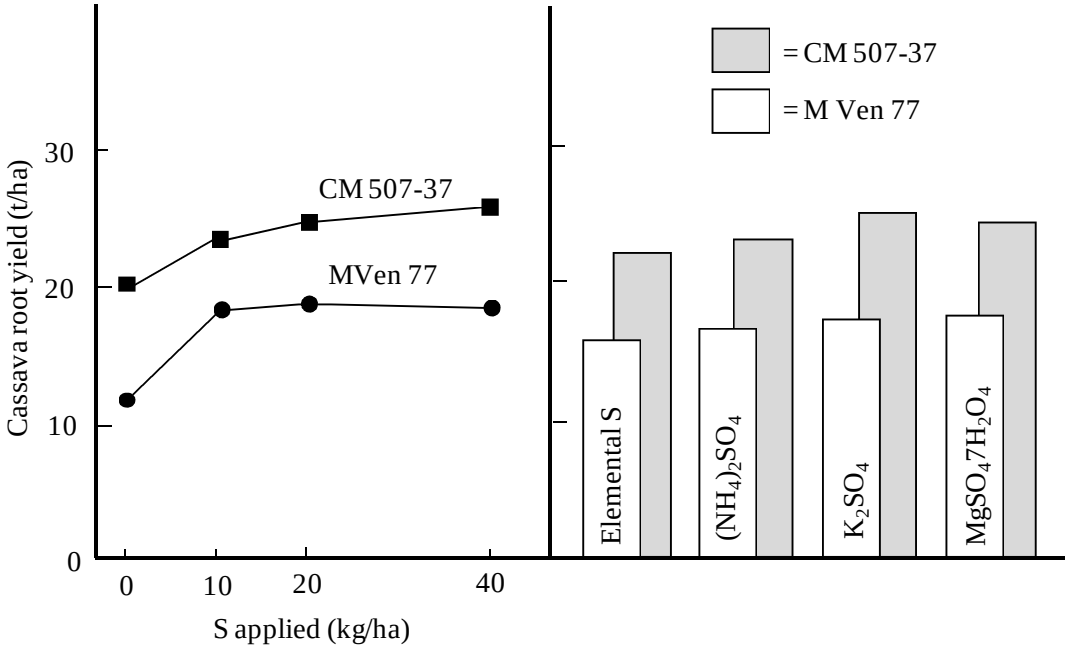

Figure 4. Effect of sources and levels of applied S on the fresh root yield of two cassava varieties grown in Carimagua-Yopare, Colombia in 1986/87.
***Source**: Howeler, 2002.*

Micronutrients

Micronutrients are absorbed by the plant in very small quantities, but are a basic component of many enzymes and thus play an essential role in most metabolic processes. There are few reports on micro-nutrient deficiencies in cassava, but these deficiencies may be more common than is generally recognized. Deficiencies of micronutrients, i.e. B, Cu, Fe, Mn and Zn, are most often observed in high pH or calcareous soils, but deficiencies of Zn have been observed in both acid and alkaline soils. Lime application to acid soils with low levels of available Zn may induce Zn deficiency, resulting in low yields and even death of young plants.

Zinc

Cassava is susceptible to Zn deficiency, especially at the early stages of growth. Symptoms of Zn deficiency appear as intervenal chlorotic spots or lines on younger leaves. When very severe, the whole leaf becomes pale green to white, leaf lobes become smaller and tend to point outward away from the stem. Oftentimes, lower leaves show necrotic white or brown spotting and the plant remains small and weak. Plants showing early symptoms of Zn deficiency may later recuperate once the fibrous root system is well established and roots become infected with mycorrhizae. If the deficiency is severe, however, plants may either die or produce very low yields.

Symptoms of severe Zn deficiency have been observed in acid soils in Colombia, Brazil, Malaysia, Thailand, Nigeria, Tanzania and Mexico, as well as in alkaline and/or calcareous soils in Colombia, Cuba, Mexico and Indonesia.

On acid soils Zn deficiency can be controlled by incorporation of ZnO or band placement of $ZnSO_4.7H_2O$ at the rate of 10-20 kg Zn/ha. Also effective are foliar applications of 1-2% $ZnSO_4.7H_2O$ or stake treatments in 2-4% $ZnSO_4.7H_2O$ solution during 15 minutes before planting.

Figure 5A shows the response of two varieties to soil application of different levels of Zn as $ZnSO_4.7H_2O$ in a very acid soil in Carimagua-Alegría, Colombia, after applying 2 t/ha of lime (CIAT, 1985). Both varieties were seriously affected by Zn deficiency in the check plots, but reached maximum yields with the application of 10 kg Zn/ha, band applied together with NPK-fertilizers at planting. **Figure 5B** shows the relation between the root yield of MVen 77 and the Zn concentration in YFEL blades at 4 MAP. A critical level of 33 ppm Zn was estimated. Broadcast application of 10-20 kg/ha of Zn as ZnO was also effective in increasing yields in acid soils (CIAT, 1978).

In high-pH soils, application of $ZnSO_4.7H_2O$ to the soil is not so effective because the applied Zn will be precipitated rather rapidly (CIAT, 1978). Foliar application or stake treatments may be more effective. When 20 cassava cultivars were planted in a high-pH (7.9), low-Zn (1.0 ppm) soil, with or without treating stakes for 15 minutes in a solution of 4% $ZnSO_4.7H_2O$ before planting, yields increased from an average 11.5 to 25.0 t/ha due to the Zn treatment (CIAT, 1985). Large varietal differences in low-Zn tolerance were observed, with some cultivars dying off completely without the Zn treatment, while others producing high yields with or without Zn.

Copper

Copper deficiency in cassava results in reduced plant height, chlorosis and curling of upper leaves and necrosis of leaf tips. Lower petioles tend to be long and droopy.

Severe Cu deficiency has been reported only in peat soils of Malaysia. A basal application of 2.5 kg Cu/ha as $CuSO_4.5H_2O$ increased yields from 4 to 12 t/ha (Chew, 1971; Chew *et al*., 1978).

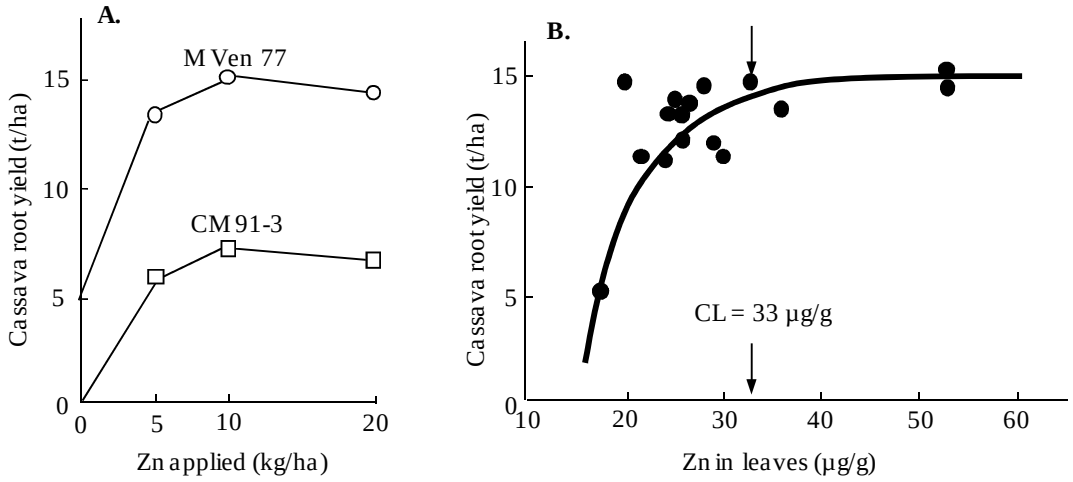

Figure 5. Root yield response of two cassava varieties to various levels of applied Zn (A), and the relation between the root yield of cv. MVen 77 and the Zn concentration in YFEL-blades at 4 months after planting in Carimagua-Alegria, Colombia (B). Arrows indicate the critical level for Zn deficiency.
***Source**: CIAT, 1985.*

Table 3. Zinc concentration of YFEL blades at 4½ months after planting and root yields of 20 cassava varieties planted with and without a stake treatment with 4% $ZnSO_4.7H_2O$ in an alkaline soil at CIAT, Palmira, Colombia.

Variety	Zn concentration in leaves with Zn treatment (ppm)	Fresh root yield (t/ha)	
		With Zn	Without Zn
MPer 176	22	3.9	0
MPer 193	19	24.9	10.7
MPer 196	17	30.5	13.8
MPer 200	22	35.4	15.0
MPer 206	20	31.1	9.0
MPer 211	20	21.9	9.2
MPer 239	20	25.9	13.0
MPer 243	24	7.6	6.5
MPer 244	25	18.0	14.1
MPer 245	23	1.0	0.6
MPer 247	22	48.7	31.3
MPer 252	26	22.8	17.2
MPer 253	20	44.9	10.7
MPer 266	20	20.4	8.1
MCol 22	21	23.3	11.2
MCol 113	25	35.3	9.4
MCo 1438	22	3.7	2.3
MVen 290	20	8.8	3.4
CM 231-188	21	47.6	23.5
CM 498-1	18	44.8	21.2
Average		**25.0**	**11.5**

***Source**: CIAT 1985.*

Iron

Iron deficient plants have smaller but normal-shaped upper leaves that are light-green, yellow or white in color. When severe, even the upper petioles are white.

Iron deficiency has been observed in calcareous soils of the Yucatan peninsula of Mexico, in northern Colombia, in Tamil Nadu state of India, in western Nakorn Ratchasima province of Thailand and along the southern coast of Java island in Indonesia. It is also commonly seen in old termite hills which tend to have a soil pH considerably higher than the surrounding area.

A practical solution is probably a stake treatment with 2-4% $FeSO_4.7H_2O$ or foliar applications of Fe-sulfate or chelates.

Manganese

Manganese deficiency is characterized by intervenal chlorosis (fish-bone pattern) of middle leaves, similar to Mg deficiency but generally not present in lower leaves. When severe, the whole leaf turns uniformly yellow, very similar to Fe deficiency or salinity.

Manganese deficiency has been observed in alkaline soils in the Cauca valley of Colombia, along the coast in NE Brazil, and in north Vietnam, near houses where lime had been used for their construction. Stake treatments before planting with $MnSO_4.4H_2O$ or foliar sprays with sulfates or chelates are probably the most practical solutions

Boron

Being a phloem immobile nutrient, B is not readily translocated to the growing points. Thus, in case of B deficiency, both the young shoots and the root system are affected. In nutrient solution, cassava plants suffering from severe B deficiency have a deformed growing point with very short internodes and small dark-green leaves. Sometimes the petioles or stem exude a brown gummy substance, which later produce brown lesions. The root system is short and stubby. In the field, however, these severe symptoms are seldom observed; instead, B deficient plants have chlorotic small spots on middle or lower leaves. Similar symptoms were also observed in north Vietnam and southern China, although the exact nature of those problems was never identified.

Some symptoms of B deficiency have been observed both in acid soils of Carimagua and Quilichao and in alkaline soils at CIAT-Palmira. Applications of 1-2 kg B/ha, band applied as Borax at time of planting, eliminated these symptoms, increased plant height, increased B concentrations in the leaves from 3 to 40 ppm, but had no significant affect on yield. Cassava seems to be much more tolerant of low B concentrations in the soil than maize or *Phaseolus* beans (Howeler *et al*., 1978).

Boron toxicity has not been observed under natural conditions, but is easily induced by excessive applications of B to the soil or in stake treatments. B toxicity causes necrosis of lower leaves. Since the element is not readily translocated to the growing points, plants generally recuperate.

Aluminum and Manganese Toxicity and Low pH

Large parts of the tropics are unproductive because the soils are too acid for most cultivated crops, and the lack of adequate roads makes the transport of lime prohibitively expensive. In these areas cassava is often the staple food because this crop is highly

tolerant of low pH and the associated high levels of Al and Mn, low levels of Ca, Mg and K, and sometimes low P and N. Cassava as a species is particularly tolerant of soil acidity and high levels of Al (Gunatilaka, 1977; CIAT, 1979; Islam *et al*., 1980), but some varietal differences in acid soil tolerance have also been observed (CIAT, 1982; 1985; Howeler, 1991).

Clear symptoms of Al-toxicity in the field are seldom observed, except that plants are small and lack sufficient vigor. Under severe Al toxicity conditions in nutrient solutions lower leaves may have intervenal chlorosis and necrotic spots. High levels of Al have an especially detrimental effect on root growth, which in turn affects nutrient and water absorption.

Plants suffering from Mn toxicity have droopy yellow bottom leaves with brown or black spots along the veins. These leaves may later fall off leaving the plant without recognizable symptoms. Mn toxicity occurs only in very acid soils high in Mn and mainly in areas of compacted soils leading to poor drainage. This enhances the solubility of Mn due to reduction processes. Mn-toxicity not only reduces the vigor of the plant tops but also seriously affects the root system. Compared with other crops, cassava is relatively tolerant of high levels of Mn. Among 13 plant species studied only three species were more tolerant (Edwards and Asher, unpublished). Among cassava cultivars considerable differences in tolerance were also observed. Mn-toxicity in cassava has been reported only in acid Ultisols and Inceptisols in Quilichao, Colombia, and in a compacted sandy clay loam soil in Thailand (Wichit Silpamaneephan, 1994). Application of lime in acid soils decreases both the concentration of Al and Mn, reducing their toxic effects.

Figure 6 shows that when increasing levels of lime were applied to a very acid soil in Carimagua, Colombia, there was a successive increase in soil pH and a decrease in exchangeable Al. Application of 6 t/ha of lime reduced the Al-saturation (i.e. exchangeable Al divided by exchangeable Al +Ca + Mg + K, all expressed in meq/100 g) from 85% to 20%. **Figure 7** shows that corn, upland rice, bean and sorghum produced very poorly without lime and required 6 t/ha of lime to reach maximum yields. On the other hand, cowpea and cassava produced still 40% of maximum yield without lime and close to maximum yields with only 2 t/ha of lime. Among cassava cultivars there are differences in their tolerance to acid soils, and highly tolerant cultivars should be selected for acid soil regions (Howeler, 1991)

In very acid (pH<4.5) and high Al (>80% Al-saturation) soils, lime application may increase cassava yields, mainly by supplying Ca and Mg as nutrients. High rates of lime, however, may induce micronutrient deficiencies, particularly Zn, resulting in decreased yield (Spain *et al*., 1975; Edwards and Kang, 1978).

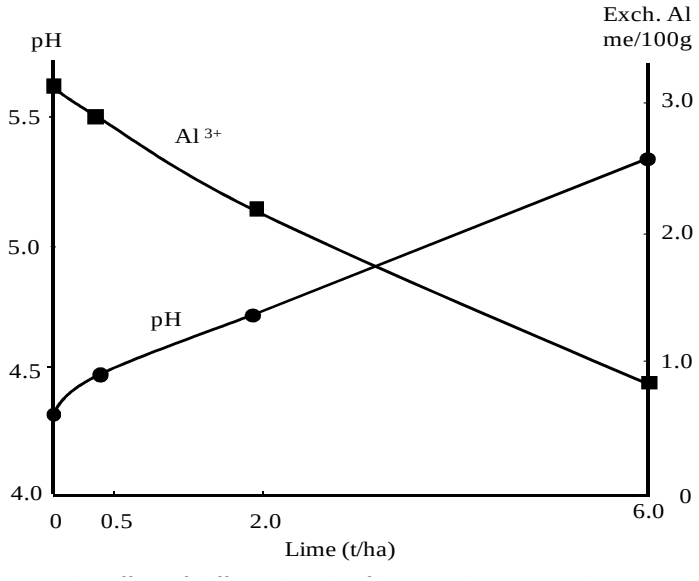

Figure 6. The effect of different levels of lime application on the pH and exchangeable Al in an acid Oxisol in Carimagua, Colombia.
Source: *Spain et al., 1975*

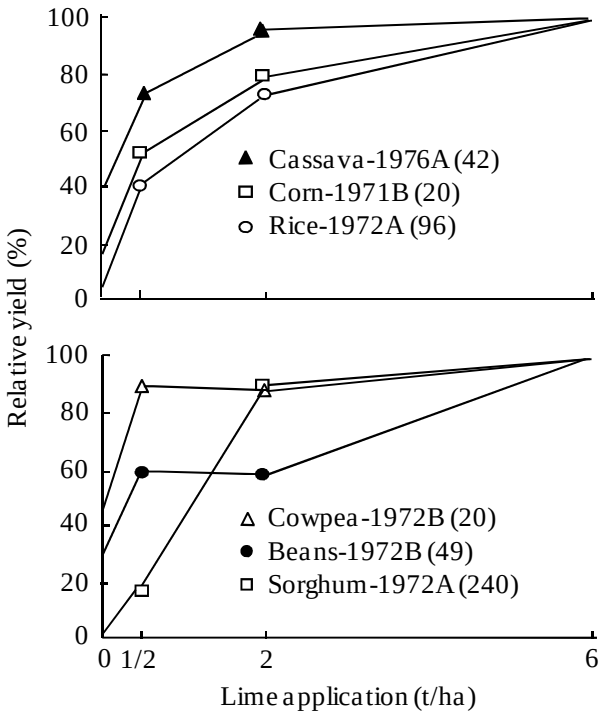

Figure 7. The effect of different levels of applied lime on the relative yield of six crops grown in Carimagua, Colombia. Numbers in parentheses indicate the number of varieties tested.
Source*: CIAT, 1978.*

Figure 8A shows that without applied Zn, cassava responded to lime applications only up to 2 t/ha, but with applied Zn there was a positive response up to 6 t/ha of lime. Analysis of cassava leaves (**Figure 8B**) confirmed that liming reduced Zn uptake and that with 6 t/ha of lime without Zn, the Zn concentration in YFEL blades dropped below the critical level of 40-50 ppm. Large varietal differences have been observed for both high-Al and low-Zn tolerance (Spain *et al*., 1975; CIAT, 1985).

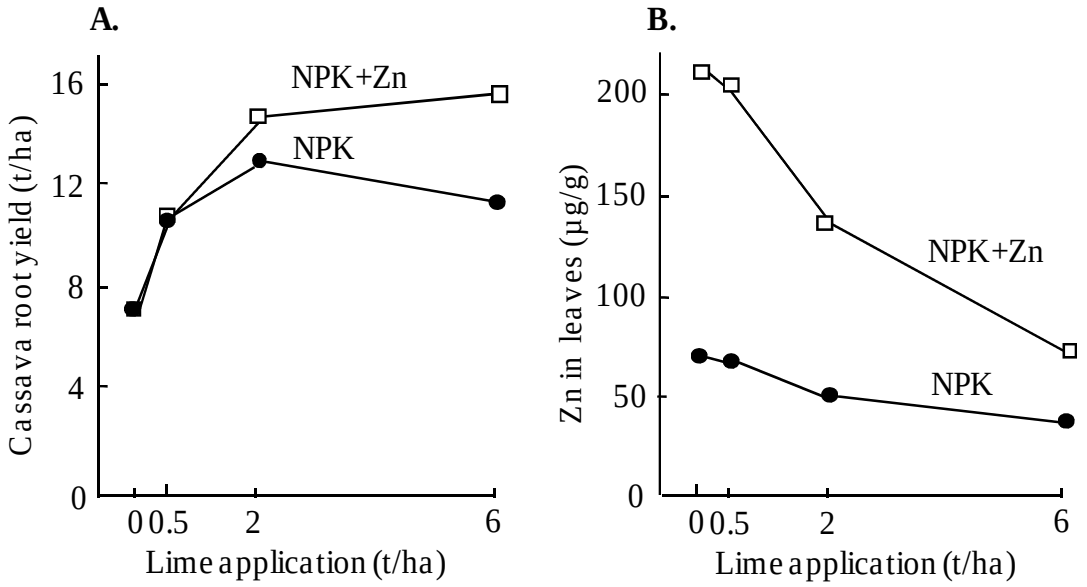

Figure 8. Effect of lime application on root yield (A) and Zn concentration in YFEL blades at 2 MAP of cassava, cv. Chirosa de Acacias, grown with and without the application of 20 kg/ha of Zn in Carimagua, Colombia.
***Source**: CIAT, 1976.*

Soil Salinity, Alkalinity and High pH

While cassava is very tolerant of soil acidity, it is quite susceptible to salinity, alkalinity and high pH. Islam (1979) showed that in nutrient solution cassava had optimum growth at pH 5.5 to 7.0 but top growth declined markedly above pH 7.5-8.0. The species was among the most tolerant of low pH and most susceptible to high pH **(Figure 9).** In natural soils high pH is generally associated with high levels of salts (salinity) and Na (alkalinity), poor drainage and minor element deficiencies. The crop usually suffers from a combination of these factors, which are difficult to study individually under field conditions. Also, salinity-alkalinity problems occur in spots in the field giving rise to extremely heterogeneous soils and highly variable plant growth. In **Figure 10** cassava root yields were related to soil pH, percent Na-saturation and soil solution conductivity. While there were significant differences among the three cultivars, root yields declined markedly above pH 8.0, above 2.5% Na-saturation and above 0.5-0.7 mmhos/cm of electrical conductivity (CIAT, 1977). Yield reductions are probably due to the combined effect of all three factors. In comparison, many other crops tolerate up to 15% Na saturation or 4 mmhos/cm conductivity.

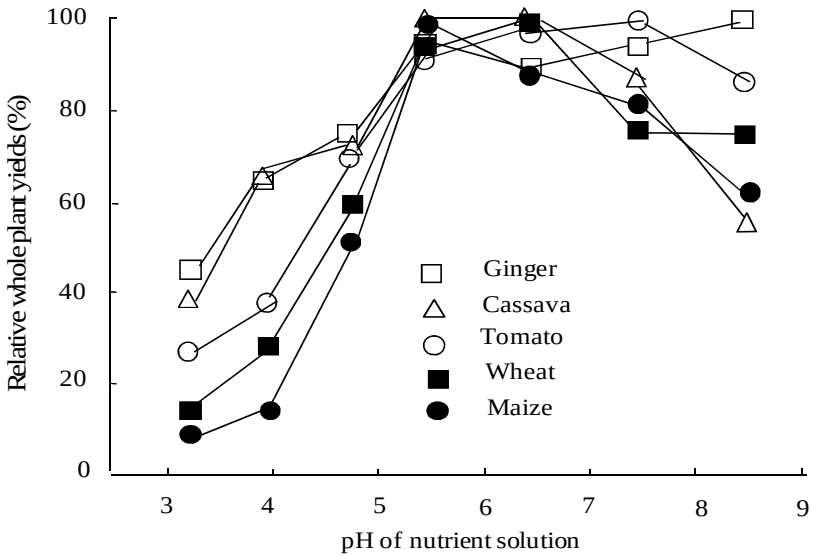

Figure 9. Relative growth response of various plant species to a series of constant pH values maintained in flowing nutrient solutions.
Source: *Islam et al., 1980.*

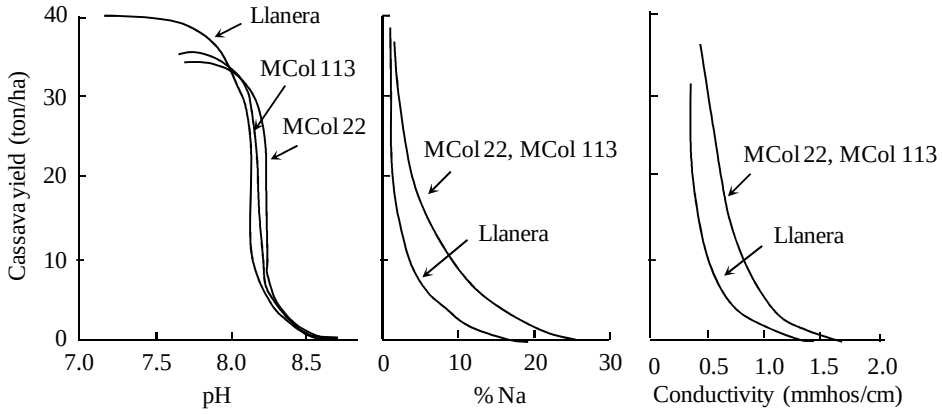

Figure 10. Relation between the root yield of three cassava varieties and soil pH, percent Na-saturation and soil solution conductivity in a saline-alkaline soil in CIAT-Colombia.
Source*: CIAT, 1985.*

Problems of soil salinity-alkalinity are very costly to resolve. Yields can be improved by applying 1-2 t/ha of elemental S or 1-2 t/ha of H_2SO_4 (CIAT, 1977), but this is seldom justified economically. Large varietal differences in tolerance have been observed, and the use of tolerant varieties is probably the most practical solution.

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CHAPTER 18

SOIL FERTILITY MAINTENANCE: ORGANIC SOLUTIONS

Reinhardt Howeler[1]

Before the advent of chemical fertilizers, farmers maintained the productivity of their soils through crop rotations with leguminous pastures (mainly in temperate climates) or by shifting cultivation (mainly in tropical climates). The latter system works well except in those areas where, due to increasing population pressure, fallow periods have decreased from 10-20 years to only 2-3 years. These short fallows are insufficient to restore fertility and yields can not be maintained. To improve the system and accelerate the restoration and maintenance of soil fertility, several systems of "planted fallows", "green manuring", "alley cropping", "cover cropping" and "intercropping" have been investigated and sometimes promoted. In most of these systems, leguminous tree or forage species are used to fix N from the air and to recycle other soil nutrient. At the same time, the organic matter input will increase the soil's water and nutrient holding capacity and stimulate microbial activity. All these systems have certain advantages and disadvantages, and farmers will have to decide which are most appropriate for their particular conditions.

This chapter reviews research conducted on the efficiency of fallows as well as on the use of grain and forage legumes for improving soil fertility and/or reducing erosion in cassava fields through intercropping, green manuring, alley cropping, mulching and cover cropping, as well as the application of animal manures with or without chemical fertilizers.

Shifting Cultivation

In many areas in the tropics farmers seldom apply any chemical fertilizers or manures to their cassava crop; instead, they try to maintain soil fertility through shifting cultivation, also known as "slash-and-burn" systems, in which, after several years of cropping, the land is returned to bush fallow for 10-20 years to let the soil rest and replenish the nutrients that were lost during the cropping cycle. However, because of rapid population growth and the consequent increase in land pressure, the fallow period has steadily been shortened while the cropping cycle and intensity have increased.

In the early 1980s a study was undertaken in the mountainous cassava growing region of Cauca Department in Colombia, to determine the effect of the length of the fallow period on the yields of subsequently grown cassava, and whether longer fallows can actually replace the use of chemical fertilizers. In this area of very poor and eroded soils, cassava is the main crop used for home consumption and for sale to small cassava starch factories. Farmers were interviewed about their cassava cropping practices and asked about the lengths of the fallow period of their plots. These plots were then separated into four groups that had had fallow periods of 1-2, 4-5, 7-10 and >15 years. Simple on-farm trials were established on each of these four categories of length-of-fallow plots with seven fertilizer treatments: no fertilizers, three levels of P fertilizers without N and K, and the

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same three P treatments combined with N and K. The trials were continued for three cropping cycles.

Figure 1 shows that the length of the fallow period had no consistent effect on cassava yields, with the shorter fallow period often producing higher yields than the longer periods. Application of only P fertilizers in general had a marked positive effect on cassava yields, but in a few cases this effect was minor or even negative. The application of all three nutrients, however, was very effective in increasing yields, and this effect was independent of the length of the previous fallow period.

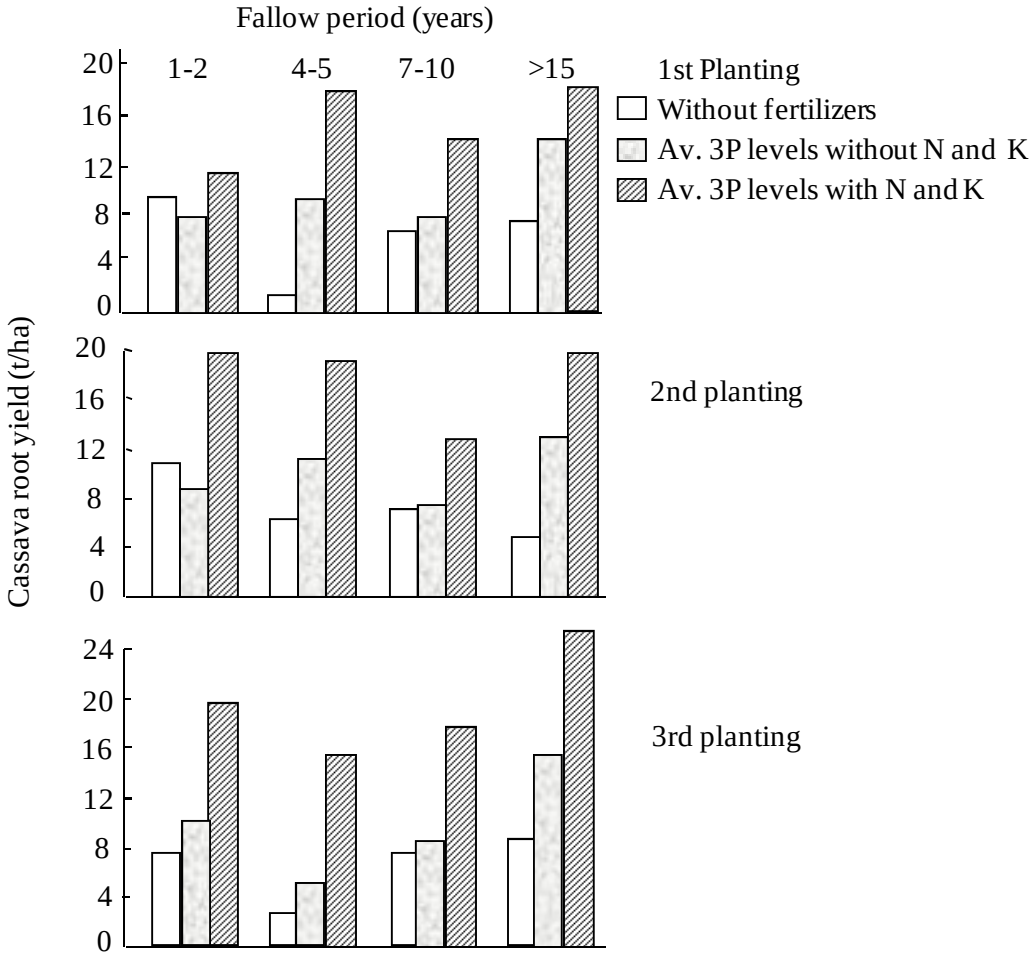

Figure 1. Effect of the length of fallow period on the yield of three consecutive cassava crops grown with various fertilizer treatments on farmers' fields near Mondomo, Cauca, Colombia. ***Source****: CIAT 1988.*

Thus, it is clear that in these very poor and degraded soils, even long periods of bush fallow were not able to fully restore soil fertility, and cassava yields remained below 8-10 t/ha. In contrast, with the application of N, P and K in chemical fertilizers cassava yields could double or triple, reaching over 24 t/ha in the third consecutive planting. In this and many similar situations, farmers could greatly increase their income if they would grow

cassava on a more permanent basis on the best and flattest land, using chemical fertilizers, while leaving the steeper and more degraded fields in permanent pasture, coffee, fruit trees or forest. Unfortunately, in many of these areas fertilizers are not readily available or the farmers don't have the knowledge or the financial resources to buy fertilizers.

Green Manures

The use of green manures to maintain or improve soil fertility is a practice that has been widely researched and promoted by soil scientists but has not been widely adopted by farmers. Green manures are usually grain or forage legumes that are planted to fix N and recycle other nutrients from the subsoil to the topsoil so as to improve the nutrient supply for the following crop. The green manures are generally cut after 3-4 months of growth and either incorporated into the soil or mulched on top of the soil before planting the following crop. However, they can also be planted as an intercrop within the main crop and slashed back and mulched after 2-3 months of growth, or planted as narrow strips alternating with strips of the main crop.

1. Green manuring of cassava with grain and forage legumes in Quilichao, Colombia

An experiment was initiated in 1983 to see whether green manures, with or without fertilizers, could restore soil productivity in a soil that had previously been cropped with cassava for five years without fertilizer inputs. Three grain legumes and five forage legumes were planted after application and incorporation of 1 t/ha of lime; they also received 250 kg/ha of banded 10-30-10 fertilizers. The check plot without green manure did not receive this basal fertilizer application. The grain legumes, cowpea, peanut and pigeon pea, were harvested after four months, and the forage legumes were cut at six months, followed by incorporation of the forages and crop residues into the soil. One month later, two cassava varieties were planted, both with and without band application of 500 kg/ha of 10-30-10 fertilizers. After the cassava harvest at 12 MAP a second cassava crop was planted, again with and without fertilizers to measure the residual effect of the green manures.

Table 1 shows the dry matter production of the green manures and their effect on soil fertility parameters before the first and second cassava crops, as well as the yields of cassava (only cv. MCol 1684) in both crop cycles. Velvet bean (*Mucuna pruriens)* and pigeon pea produced the greatest amount of DM, followed by *Indigofera* and peanut. Incorporation of the green manures had only a minor effect on soil fertility, except that kudzu (*Pueraria phaseoloides*) increased levels of both soil P and K, while peanut increased mainly soil K. Some increase in soil P and K was due to the band application of 250 kg/ha of 10-30-10 at time of planting the green manures; this was not applied to the check plots without green manures.

During the first cassava cycle, all green manures increased yields when no fertilizers had been applied to cassava, while some green manures increased and others decreased yields when fertilizers had been applied. Peanut, pigeon pea and kudzu were the most effective in the absence of fertilizers, while kudzu and peanut were most effective in the presence of fertilizers. Application of fertilizers in the absence of green manures increased cassava yields from 16.9 to 31.9 t/ha, while incorporation of green manures increased cassava yields at most to 29.3 t/ha with the use of peanut. Velvet bean and cowpea were not very effective in increasing yields in the absence of fertilizers, and

actually decreased yields in the presence of fertilizers. In case of velvet bean, cassava growth was clearly stunted, possibly due to an allelopathic effect. Soil analyses before the second cassava crop indicate that the fertilizers applied to cassava had little residual effect on soil fertility, possibly because of the high cassava yields (up to 41 t/ha) obtained. Peanut and *Indigofera* had increased soil P, while soil K was very low for all treatments. There was no apparent residual effect of the green manures on soil K.

Table 1. Dry matter production of various green manures and the effect of their incorporation on soil fertility (A), and on yield of cassava, cv. MCol 1684, grown without or with application of chemical fertilizers[1] (B) in 1983 and 1984 in Quilichao, Colombia.

A.	DM	Soil fertility in 1983 [2]				Soil fertility in 1984 [2]	
Green manure treatments	green manures (t/ha)	pH	OM (%)	P (ppm)	K (me/100 g)	P (ppm)	K (me/100 g)
1. no green manures	-	4.1	5.5	3.8	0.10	3.6	0.08
2. cowpea	0.45	4.0	5.5	5.2	0.12	5.5	0.08
3. peanut	1.75	4.1	5.9	5.1	0.14	6.2	0.09
4. pigeon pea	1.95	4.1	6.0	4.6	0.13	6.6	0.07
5. velvet bean	1.95	4.1	5.6	5.5	0.12	5.8	0.08
6. *Zornia latifolia*	0.55	4.1	5.6	5.2	0.12	5.1	0.07
7. *Centrosema pubescens*	0.90	4.1	5.9	4.6	0.11	5.0	0.08
8. *Indigofera hirsute*	1.90	4.1	5.8	5.5	0.13	6.7	0.08
9. *Pueraria phaseoloides*	1.00	4.1	5.6	7.7	0.15	5.4	0.08

B.	Cassava fresh root yield (t/ha)			
	1983/84		1984/85 [3]	
Green manure treatments	w/out fertilizers	with fertilizers	w/out fertilizers	with fertilizers
1. no green manures	16.9 c	31.9 abcd	13.6 b	31.4 bcd
2. cowpea	18.9 bc	26.5 cd	19.5 ab	32.2 abcd
3. peanut	29.3 a	39.0 a	24.6 a	30.0 cd
4. pigeon pea	28.6 a	33.8 abc	18.8 ab	38.9 a
5. velvet bean	19.9 bc	23.6 d	18.9 ab	31.9 abcd
6. *Zornia latifolia*	24.1 abc	41.1 a	22.3 ab	28.6 d
7. *Centrosema pubescens*	25.1 abc	36.7 ab	15.2 ab	40.0 a
8. *Indigofera hirsute*	25.7 ab	29.7 bcd	12.6 b	34.8 abcd
9. *Pueraria phaseoloides*	26.9 ab	40.4 a	13.7 b	37.3 abc
Average	**23.9 b**	**33.6 a**	**17.7 b**	**33.9 a**

[1] band application of 500 kg/ha of 10-30-10 (N-P_2O_5-K_2O) fertilizer with both cassava crops
[2] average of with and without fertilizers
[3] residual effect of green manures planted in 1983 on cassava yields in 1984/85.

In the second cassava crop, fertilizer application in the absence of green manures increased yields from 13.6 to 31.4 t/ha. In the absence of fertilizers, peanut and *Zornia* increased yields markedly, but only that of peanut was statistically significant. In the presence of fertilizers the effect of green manures was statistically significant. *Centrosema* and pigeon pea increased cassava yields significantly, while *Zornia* and peanut slightly decreased yields.

From this experiment it may be concluded that cassava yields were increased most markedly by the application of fertilizers, but that incorporation of green manures also helped to increase yields, especially when no fertilizers were applied to cassava. Without fertilization, peanut, pigeon pea and kudzu were most effective in the first planting, while peanut and *Zornia* were most effective in the second crop. With application of fertilizers, kudzu, *Zornia* and peanut were most effective in the first planting, while *Centrosema*, pigeon pea and kudzu were most effective in the second crop.

2. Green manuring of cassava with grain and forage legumes in Media Luna, Colombia

Another experiment was planted in Media Luna on the north coast of Colombia, in very sandy soils, low in OM and nutrients. Since previous trials had shown that responses to chemical fertilizers were not as great as might be expected, a green manure trial was established to determine whether green manures could increase yields both in the presence and absence of chemical fertilizers. The green manures were cut and mulched after three months and two cassava cultivars, MVen 25 and MCol 2215, were planted, either without or with band application of 500 kg/ha of 15-15-15 fertilizers. One check plot with weeds removed and one with native weeds cut and mulched were also included. The native weeds consisted mainly of tall grasses and creeping legumes.

Table 2 shows that peanut, *Indigofera* and native weeds had the highest DM yields, while *Crotalaria juncea* was least productive and had only a minor effect on soil fertility. Green manures (including native weeds) slightly increased soil OM. Mulching of *Canavalia* resulted in highest levels of soil P, Ca and K., while native weeds also increased Ca and Mg, but had little effect on P and K.

Table 2. Dry matter production of native weeds and green manures and the effect of mulching on soil fertility and on the yield of cassava, cv. MVen 25, grown without and with application of fertilizers in sandy soils of Media Luna, Colombia.

Green manure treatments	DM green manures (t/ha)	At time of planting cassava						Cassava root yield (t/ha)	
		pH	OM (%)	P (ppm)	Ca ← (meq/100 g) →	Mg	K	without fertilizer	with fertilizer[1)]
1. no green manures	-	5.2	0.70	6.4	0.43	0.11	0.04	19.5	34.3
2. native weeds	4.73	5.5	0.82	4.6	0.54	0.18	0.06	34.4	30.7
3. cowpea	2.93	5.3	0.77	5.9	0.52	0.16	0.07	27.6	32.5
4. peanut	6.56	5.3	0.97	6.1	0.45	0.13	0.07	32.0	24.8
5. pigeon pea	3.93	5.1	1.15	8.4	0.54	0.17	0.07	30.2	29.7
6. velvet bean	2.50	5.5	0.80	5.1	0.47	0.13	0.05	31.9	34.8
7. *Crotalaria juncea*	1.71	5.3	0.85	5.7	0.46	0.13	0.06	24.6	32.6
8. *Canavalia ensiformis*	3.29	5.0	0.85	8.0	0.56	0.17	0.09	34.0	32.9
9. *Indigofera hirsuta*	6.00	5.2	0.82	6.1	0.49	0.14	0.06	30.9	34.8
Average								**29.4**	**32.3**

[1)] with 500 kg/ha of band applied 15-15-15 fertilizers

Application of 500 kg/ha of 15-15-15 fertilizers in the absence of green manures increased cassava yields from 19.5 to 34.3 t/ha. Similar yields were obtained by the mulching of native weeds or *Canavalia* without application of fertilizers. All green manures markedly increased cassava yields when no fertilizers were applied, but *Crotalaria juncea* was least effective. In the presence of fertilizers, green manuring had no beneficial effect.

Thus, it may be concluded that in the sandy soils of Media Luna, application of 3-6 t/ha of dry mulch of green manures had similar beneficial effects as the application of chemical fertilizers. Of the green manures tested, *Canavalia ensiformis* and native weeds were most effective, while *Crotalaria juncea* was least productive and least effective in increasing cassava yields. Since cassava produced high yields when mulched with 3-6 t/ha of weeds or green manures even though the soil-K level remained far below the critical level of 0.15 meq/100 g, it appears that K, leached down the profile from the decomposing mulch, was immediately absorbed by cassava roots without increasing the level of exchangeable K in the soil In addition, the mulch may have had other beneficial effects.

Later studies in Media Luna (CIAT, 1994, 1995, 1996; Cadavid *et al*., 1998) have indicated that application of large amounts (12 t/ha) of dry mulch of *Panicum maximum* not only supplied plant nutrients, mainly K, Ca, Mg and inorganic-N, but also helped to maintain soil moisture and reduce the temperature of the surface soil. In the latter study, mulch application during eight consecutive years significantly increased cassava root and top biomass, increased root dry matter content while reducing its yearly variation, and decreased root HCN, particularly in the absence of fertilizers. Cassava yields declined in the absence of fertilizers and mulch, but increased over the years when either mulch alone or mulch and fertilizers were applied. Over the years, both the application of mulch and that of fertilizers increased the soil P and K levels, while without mulch soil pH decreased. The effect of fertilization was more pronounced in the absence of mulch.

2. Green manuring of cassava with forage legumes in Pluak Daeng, Thailand

An experiment on the use of forage legumes as green manures to maintain soil fertility in sandy clay soils was also conducted in Pluak Daeng in Rayong province of Thailand in 1988/89. The green manures were planted in the beginning of the wet season (May/June) and after 3-4 months the above-ground parts were cut and incorporated into the soil before planting cassava in the mid to late wet season (Aug/Sept). Cassava did not receive any fertilizers, except in one of the two treatments without green manures which received 100 kg N and 50 K_2O/ha. Cassava was harvested after about eight months at the start of the next wet season. The experiment was repeated in a similar fashion in 1989/90 and 1990/91.

Table 3 shows the productivity of the green manures and their effect on cassava yields during the three years of testing. There was a significant effect of green manure application on cassava yields in the first two years, but the effect was not significant in the last year. *Crotalaria juncea* and *Canavalia ensiformis* were the most productive species and the most effective in recycling nutrients (Tongglum *et al*., 1992), while incorporation or mulching of *Crotalaria juncea* usually resulted in the highest cassava yields; these yields were similar to those obtained with chemical fertilizers. Other promising species were *Mucuna fospeada* and *Canavalia ensiformis*. Nevertheless, in the first two years cassava

yields were extremely low because cassava could only be planted late in the rainy season after the green manures had been incorporated or mulched. As such, cassava suffered from drought stress during much of the growth cycle. In the third year, cassava was not harvested until August 1991 (11 months), resulting in much higher yields, but there was no significant response to green manure applications.

Table 3. Green manure productivity and their effect on cassava yields in three experiments conducted in Pluak Daeng, Rayong, Thailand.

	DM green manures (t/ha)			Cassava fresh root yield (t/ha)		
Green manure treatments [1)]	1988/89	1989/90	1990/91	1988/89	1989/90	1990/91
no green manure, no fertilizers	-	-	-	3.21 cd	5.75 bcd	16.36
Sesbania rostrata	9.71 b	3.46 b	9.91 b	9.29 a	5.37 bcd	15.04
Sesbania speciosa	2.58 ef	2.15 b	9.73 b	5.61 abcd	4.46 cd	17.52
Sesbania aculeata	4.20 dc	2.54 b	7.58 b	5.19 bcd	4.42 cd	13.23
Crotalaria juncea	13.46 a	6.88 a	24.79 a	9.04 ab	8.83 a	17.29
Crotalaria mucronata CIAT 7790	6.77 c	2.86 b	10.36 b	6.71 abc	5.17 bcd	11.77
Crotalaria spectabilis	5.49 cd	2.98 b	12.75 ab	5.81 abcd	3.96 d	17.64
Canavalia ensiformis	6.63 c	6.96 a	24.79 a	5.37 bcd	7.00 abc	14.67
Indigo	6.36 c	3.21 b	10.94 b	5.37 bcd	5.08 bcd	16.61
Mucuna fospeada	5.66 cd	2.70 b	10.74 b	5.21 bcd	6.08 abcd	16.45
pigeon pea (from ICRISAT)	2.11 f	3.46 b	2.29 b	2.06 d	4.50 cd	14.79
no green manure, with fertilizers [2)]	-	-	-	8.75 ab	7.71 ab	17.04
F-test	**	**	**	**	*	NS

[1)] green manures were planted in May/June, cut in Aug/Sept and cassava was planted in Oct, harvested after 8-9 months in the first two years and after 11 months in the third year.
[2)] 100 kg N and 50 K_2O/ha; no fertilizers to cassava in the green manure treatments.

Analyses of soil samples taken before planting and after harvest of cassava indicate that green manures had no significant effect on pH, OM, and available P or exchangeable K (CIAT, 1992). In all treatments, soil pH gradually decreased from 6.6 to 5.5, OM decreased slightly from 1.0 to 0.8%, P was quite variable, while exchangeable K decreased from 0.24 to 0.08 meq/100 g.

A similar experiment was conducted for three years (1991-1994) in an adjacent field in Pluak Daeng using six green manure species. These were again planted in the early wet season (May/June), cut after three months, and (in subplots) either mulched on the soil surface or incorporated into the soil with a hand tractor. In the mulched subplots cassava was planted without further land preparation. Cassava was planted in the mid to late rainy season (Aug/Sept) and harvested after 9-10 months. For comparison, two additional plots without green manures were planted at the more traditional planting time at the start of the rainy season (May/June); these were also harvested after 9-10 months. At both planting times one of the two check plots without green manures received 100 kg N and 50 K_2O/ha

Table 4 shows that planting in the early rainy season resulted in much higher cassava yields than planting towards the end of the rainy season. Application of NK

fertilizers increased yields but not significantly. Among the six green manures, *Crotalaria juncea* was consistently the most productive specie, while *Sesbania rostrata* was the least productive. *Crotalaria juncea*, either when mulched or incorporated, also produced the highest cassava yields. While these yields were higher than those planted in Sept with fertilizers, they were not significantly different from yields obtained without fertilizers when cassava was planted in the early wet season, and they were considerably lower than those obtained with fertilizer and planted in May/June.

Soil analyses again indicate that incorporation or mulching of green manures had no significant effect on soil fertility parameters. This indicates that nutrients leached down from the decomposing green manures were directly absorbed by cassava roots without having a long-term effect on soil fertility.

Table 4. Effect of cassava planting time, fertilization and green manuring on green manure production and cassava yields in Pluak Daeng, Thailand. Data are average values for three cropping cycles, 1991/92, 1992/93 and 1993/94.

	DM green manures (t/ha)		Cassava fresh root yield (t/ha)		
Green manure treatments	incorporated	mulched	incorp.	mulched[1)]	Average
no green manure, June planting, no fertilizer	-	-	11.06	9.13	10.09 ab
no green manure, June planting, with fertilizer[2)]	-	-	13.69	13.17	13.43 a
no green manure, Sept planting, no fertilizer	-	-	5.76	4.45	5.11 cd
no green manure, Sept planting, with fertilizer[2)]	-	-	6.49	5.57	6.03 cd
Sesbania rostrata, Sept planting no fertilizer	0.84	1.11	5.25	3.63	4.44 d
Mucuna fospiada, Sept planting, no fertilizer	3.08	3.78	7.44	9.41	8.42 bc
Crotalaria juncea, Sept planting, no fertilizer	6.22	6.92	9.92	10.47	10.20 ab
Canavalia ensiformis.,Sept planting, no fertilizer	3.27	3.64	6.83	6.94	6.88 bcd
cowpea, Sept planting, no fertilizer	2.10	2.97	7.40	4.61	6.00 cd
pigeon pea, Sept planting, no fertilizer	3.10	3.57	9.31	6.17	7.74 bcd
Average	**3.10**	**3.66**	**8.32 A**	**7.36 A**	

F-test for cassava yield: main plots (A) NS; green manure treatments (B) **; AxB NS

[1)] cassava planted without land preparation
[2)] 94 kg N and 50 K_2O/ha

From these two experiments conducted in Pluak Daeng it was concluded that among the green manures tested, *Crotalaria juncea* was the most productive and the most effective in increasing cassava yields; that incorporation resulted in slightly higher yields than mulching (not statistically significant); and that some green manures were as effective or even more effective than chemical fertilizers in increasing yield. However, under the climatic conditions of Thailand, which has a 6-month dry season, the traditional use of green manures is impractical, since the better part of the rainy season is used for production of green manures, while the following cassava crop produces low yields due to drought stress in the dry season.

3. Alternative management of green manures in Rayong, Thailand

To overcome some of the above mentioned constraints, alternative management practices were tested in a green manure experiment conducted at Rayong Field Crops Research Center in Rayong, Thailand, from 1994 to 1999, using *Crotalaria juncea*, *Canavalia ensiformis*, pigeon pea and cowpea as the green manures. Three methods of green manure management were tested: a) green manures were intercropped with cassava, pulled out at two months after planting (MAP) and mulched between cassava rows; b) green manures were interplanted into a mature cassava stand at 7 MAP; they were pulled up and mulched at the time of the next cassava planting; or c) green manures were grown as a conventional green manure crop before being pulled up at 3-4 MAP and mulched, after which cassava was planted without further land preparation and left to grow for 18 months. The last method resulted in a 21 month crop cycle.

The results, shown in **Table 5**, indicate that *Crotalaria juncea* usually had the highest DM production, followed by pigeon pea or cowpea. Pigeon pea was particularly productive as a green manure crop when interplanted at 7 MAP, in which case the green manure remained in the field during the dry season. Because of their high DM production, *Crotalaria* and pigeon pea were the most effective in recycling nutrients.

In the first cycle almost all green manure treatments increased cassava yields compared with the check without green manure (T_1); however, these yields were still below those obtained with a higher fertilization rate (T_2). In the second and third cycle, intercropping or interplanting of the green manures had still no significant effect on cassava yields, which were again considerably below that obtained with a higher rate of fertilization (T_2). Leaving cassava grow for 18 months after a conventional green manure crop (T_{11}-T_{14}) resulted in very high cassava yields while having little effect on root starch content. This may be an effective way for farmers to reduce production costs, since land preparation, weeding and harvesting is done only once in two years, while total production from three 21 month-cycles was similar or higher than that of five 1-year cycles (**Table 5**). However, using a higher rate of fertilization without green manures still produced the highest cassava yields.

Again, there were no consistent effects of any of the green manure treatments on soil pH, OM, available P or exchangeable K. Thus, while green manuring may have some short-term benefits in terms of crop productivity, the long-term effects on soil fertility are not very clear. Whenever labor is scarce, such as in Thailand, farmers will probably prefer to maximize their yields through the use of chemical fertilizers.

Nevertheless, Paisarncharoen *et al.* (1990) reported that incorporation of vegetative cowpea (Tita-3) increased significantly the yield of the following cassava crop during five consecutive years in Khon Kaen in northeast Thailand. Incorporation of *Crotalaria juncea* also increased yields, but not significantly, while pigeon pea had little beneficial effect (Sittibusaya *et al*., 1995).

Table 5. Effect of fertilizer application, three alternative green manure practices and four different species on green manure production and nutrient content, as well as on the yield of cassava, cv. Rayong 90, grown for three consecutive cropping cycles at Rayong Field Crops Research Center in Thailand from 1994 to 1999.

Treatments[1)]	DM green manures (t/ha)		Nutrient content of green manures (kg/ha)						Cassava yield (t/ha)				
			N		P		K						
	1st[2)]	2d	1st	2d	1st	2d	1st	2d	1st cycle	2d cycle	3d cycle	Av.	Σ5 years[3)]
1. Cassava without GM, 156 kg/ha 13-13-21	-	-	-	-	-	-	-	-	17.56	30.06	14.39	20.67	103.3
2. Cassava without GM, 467 kg/ha 13-13-21	-	-	-	-	-	-	-	-	29.78	40.39	21.42	30.53	152.6
3. C+*Crotalaria juncea*, mulched at 2 months	1.92	4.74	44.7	94.9	3.0	12.7	27.6	31.1	23.75	29.19	14.02	22.32	111.6
4. C+*Canavalia* mulched at 2 months	0.94	1.84	20.1	51.7	2.4	6.6	14.6	25.9	26.94	27.75	15.50	23.40	117.0
5. C+pigeon pea, mulched at 2 months	1.09	2.09	27.0	48.7	2.2	6.7	12.5	19.0	21.39	26.97	14.47	20.94	104.7
6. C+cowpea, mulched at 2 months	-	2.77	-	53.7	-	7.2	-	27.1	20.28	18.75	11.31	16.78	83.9
7. C+*Crot. juncea*, planted at 6-7 months	9.89	1.15	262.1	21.7	23.7	4.6	102.9	7.4	8.75	31.44	14.97	18.39	91.9
8. C+*Canavalia*, planted at 6-7 months	1.54	0.65	36.6	16.0	4.1	3.1	28.0	8.2	22.83	24.17	12.94	19.98	99.9
9. C+pigeon pea, planted at 6-7 months	8.92	2.32	221.7	45.5	20.0	7.3	108.8	15.9	15.86	28.81	14.27	19.65	98.2
10. C+cowpea, planted at 6-7 months	-	0.72	-	14.2	-	2.9	-	7.6	17.25	27.02	14.77	19.68	98.4
11. *Crot. juncea* GM, cut at 2-3m, C 18 months	1.44	4.36	39.9	79.9	3.6	17.7	14.7	31.6	46.17	49.04	36.94	44.05	132.1
12. *Canavalia* GM, cut at 2-3m, C 18 months	0.93	1.41	18.4	45.7	2.3	7.2	15.8	17.2	42.98	43.81	34.14	40.31	120.9
13. pigeon pea GM, cut at 2-3m, C 18 months	1.05	2.68	25.6	68.7	2.3	13.2	12.8	21.7	38.81	45.97	37.00	40.59	121.8
14. cowpea GM, cut at 2-3m, C 18 months	-	2.92	-	68.2	-	12.6	-	31.0	38.86	46.32	30.22	38.47	115.4

[1)] C = cassava; GM = green manure

In T3-T14 cassava received 156 kg 13-13-21/ha (like T1).

In T3-T6 cassava was intercropped with 1 row of green manure, which was pulled out and mulched at 2 MAP; cassava was harvested at 11 months for a total crop cycle of 12 months.

In T7-T10 the green manures were interplanted in the cassava stand at 7 MAP; they remained after the cassava harvest and were pulled up and mulched at time of the next cassava planting; cassava was harvested at 11 months for a total crop cycle of 12 months.

In T11-T14 the green manures were planted, pulled out and mulched at 3-4 months, after which cassava was planted and remained in the field for 18 months for a total crop cycle of 21 months.

In the first cycle, T6, T10 and T14 had *Mucuna pruriens* as the GM, but this species did not germinate well and was replaced by cowpea in the 2d and 3d cycle.

[2)] 1st and 2d refer to the first two cropping cycles

[3)] For T1-T10 estimated from the average yields in the first three years; for T11-T14 actual yields during the three crop cycles completed in slightly over 5 years

4. Long-term economic effect of green manures in Khaw Hin Sorn, Thailand

A new trial was initiated in Kasetsart University's Khaw Hin Sorn station in Chachoengsao province of Thailand in 2002 in order to determine the potential benefits of green manures planted as intercrops between cassava rows. In this case, the green manures were planted one month after the planting of cassava (to give cassava a competitive edge) and were pulled out and mulched two months later. **Table 6** shows the effect of annual planting of green manures on cassava yields during five consecutive cropping cycles. While the planting of some green manures produced slightly higher cassava yields in some years, on average none had a beneficial effect on yield. It was expected that green manures would improve both the physical and chemical characteristics of the soil, resulting in higher cassava yields, especially in these very light-textured soils that have very little organic matter (1-2%). However, the data indicate that even after five years there was still no beneficial effect of green manuring (as an intercrop) on cassava yields. *Canavalia ensiformis* and mungbean were less competitive than *Mucuna sp.* and *Crotalaria juncea*. *Mucuna* tends to climb on top of cassava plants, and is therefore not suitable as an intercropped green manure. Highest yields were obtained with the application of the high rate of 469 kg/ha of 15-7-18 and without green manures.

Table 6. Effect of green manures and/or chemical fertilizers on the root yield of cassava, cv. KU 50, planted for five consecutive years at Khaw Hin Sorn Research Station in Chachoengsao,Thailand from 2002/03 to 2006/07.

	Cassava yield (t/ha)					
Treatments[1)]	1st year	2nd year	3rd year	4th year	5th year	**Av.**
1. Check without GM; 25 kg/rai 15-7-18	46.45	26.28	32.48	36.08	18.86	**32.03**
2. *Crotalaria juncea;* 25 kg/rai 15-7-18	36.58	20.83	29.26	31.19	19.03	**27.38**
3. *Canavalia ensiformis;* 25 kg/rai 15-7-18	40.35	27.07	31.16	29.79	19.00	**29.47**
4. Pigeon pea ICPL 304; 25 kg/rai 15-7-18	38.23	24.18	31.86	30.79	19.64	**28.94**
5. Cowpea CP 4-2-3-1; 25 kg/rai 15-7-18	38.54	21.66	32.12	32.06	20.76	**29.03**
6. *Mucuna;* 25 kg/rai 15-7-18	36.73	21.17	28.58	32.09	16.45	**27.00**
7. Mungbean; 25 kg/rai 15-7-18	40.07	25.08	33.49	36.38	16.51	**30.31**
8. Check without GM; 75 kg/rai 15-7-18	43.44	32.16	37.78	34.51	27.56	**35.29**

[1)] GM = green manures; 1 ha = 6.25 rai

Source: *S. Jantawat, personal communication.*

While intercropped green manures may actually reduce cassava yields by competing with cassava for light, water and nutrients, they also compete with the local weeds, thus reducing the competition from weeds. This will reduce the normal cost of weed control. During the 5th year this reduced cost of weed control more than compensated for the additional costs of the green manure seed and the labor involved in planting and cutting back the green manures, as shown in **Table 7**. Thus, the use of green manures actually reduced the total cost of production as compared with the check without green manures. **Table 8** shows the average root yields and starch content, as well as the gross income, production costs and net income. The highest net income was obtained with the use of the high rate of chemical fertilizers, followed by the lower rate, both without green manures.

Table 7. Estimated cost of production of treatments in the green manure experiment conducted at Khaw Hin Sorn Research Station in Chachoengsao, Thailand in 2006/07 (5th year).

	Production costs for 5th year (baht/rai) [1]							
Treatments[1]	Land prepar.	Planting cassava	Fert.+ applic.	Weed control	GM planting+ harvest	GM seed	Cassava harvest+ transport	Total
1. Check without GM; 25 kg/rai 15-7-18	450	200	400	620	-	-	1,147	2,817
2. *Crotalaria juncea;* 25 kg/rai 15-7-18	450	200	400	220	220	150	1,157	2,797
3. *Canavalia ensiformis;* 25 kg/rai 15-7-18	450	200	400	220	220	150	1,155	2,795
4. Pigeon pea ICPL 304; 25 kg/rai 15-7-18	450	200	400	220	220	150	1,194	2,834
5. Cowpea CP 4-2-3-1; 25 kg/rai 15-7-18	450	200	400	220	220	170	1,262	2,922
6. *Mucuna;* 25 kg/rai 15-7-18	450	200	400	220	220	150	1,000	2,640
7. Mungbean; 25 kg/rai 15-7-18	450	200	400	220	220	120	1,003	2,613
8. Check without GM; 75 kg/rai 15-7-18	450	200	1,000	620	-	-	1,676	3,946

[1] Costs: land preparation : baht 400/rai; herbicide application: 100/rai
planting cassava: 200/rai; hand weeding (2x): 400/rai
15-7-18 fertilizers: 600/50 kg; planting + harvesting GM: 220/rai
fertilizer application: 100/rai; harvest cassava: 180/ton
Glyphosate (500 ml/rai): 120/rai; transport cassava : 200/ton
1 US$ is about 40 Thai baht

Table 8. Effect of green manures and/or chemical fertilizers on the average root yield and starch content of cassava, cv. KU 50, as well as the gross and net income during five consecutive years of cassava cropping at Khaw Hin Sorn Research Station in Chachoengsao, Thailand from 2002/03 to 2006/07.

Green manure treatments	Root yield (t/ha)	Starch content (%)	Gross income[1]	Production costs[2]	Net income
			----------('000 baht/ha)----------		
1. Check without GM; 25 kg/rai 15-7-18	32.03	24.2	37.68	17.94	19.94
2. *Crotalaria juncea;* 25 kg/rai 15-7-18	27.38	23.7	32.28	16.38	15.90
3. *Canavalia ensiformis;* 25 kg/rai 15-7-18	29.47	24.2	34.86	16.94	17.92
4. Pigeon pea ICPL 304; 25 kg/rai 15-7-18	28.94	23.6	34.04	16.83	17.21
5. Cowpea CP 4-2-3-1; 25 kg/rai 15-7-18	29.03	23.2	34.08	17.02	17.06
6. *Mucuna;* 25 kg/rai 15-7-18	27.00	24.3	32.14	16.23	15.91
7. Mungbean; 25 kg/rai 15-7-18	30.31	23.9	35.86	17.00	18.86
8. Check without GM; 75 kg/rai 15-7-18	35.29	24.4	42.39	22.04	20.35

[1] GM = green manure; all green manures were planted between cassava rows one month after planting cassava and were pulled out or cut off two months later and mulched; 1 ha = 6.25 rai.

From these various green manure trials it can be concluded that the planting of green manures can increase cassava yields in areas with a relatively long wet season or a

bimodal rainfall distribution, especially when no fertilizers are applied. However, in areas with a single and relatively short wet season the planting of green manures before incorporation or mulching, and before cassava planting may actually decrease cassava yields due to inadequate rainfall during the cassava growth cycle. In that case, leaving cassava in the ground for another year may be the most economic solution. Interplanting the green manures within a mature cassava stand at 7-8 MAP and incorporating the green manure before the next cassava planting may be another solution, while intercropping at time of cassava planting, or shortly thereafter, usually resulted in excessive competition with cassava.

The effectiveness of particular green manure species seems to vary a lot depending on their adaptation to particular soil and climatic conditions. Among the best grain legumes were peanut, pigeon pea and cowpea, and among forage legumes the most effective were *Crotalaria juncea* (mainly in slightly acid to neutral soils), and *Canavalia ensiformis*, *Zornia latifolia* and *Pueraria phaseoloides* (mainly in acid soils). Also, within each species there are many different ecotypes, which may vary in their particular adaptation and productivity. In some cases, the mulching of native weeds may be as effective as planting green manures.

In practically all trials, highest cassava yields were obtained by using chemical fertilizers rather than green manures, and in many cases this would be the most economic practice. In the absence of fertilizers, green manures may increase cassava yields, but they seldom seem to have a long-term beneficial effect on soil fertility.

Cover Crops

Cover crops are usually perennial forage legumes that are planted to fix N and recycle soil nutrients in order to improve soil fertility, and to prevent serious soil erosion on sloping land. Annual crops may be planted in individual planting holes or in strips where the cover crop has been incorporated or killed with herbicides. Several experiments have been conducted in Colombia and Thailand to see whether cover crops can improve cassava yields and/or reduce erosion when cassava is grown on slopes.

1. Cover cropping of cassava with forage legumes in CIAT-Quilichao, Colombia

Two experiments were established side-by-side on nutrient depleted soil in CIAT-Quilichao, one receiving no fertilizers and the other receiving a band application of 500 kg/ha of 10-20-20 fertilizers. Weeds were removed by hoe and two cassava varieties, MCol 1684 and CM507-37, were planted without further land preparation at a spacing of 0.8 x 0.8 m; six forage legumes were interplanted between cassava. Besides the check plot without cover crops there were two additional treatments, one in which native weeds were slashed and mulched on the soil surface, and one in which the weeds were sprayed with Paraquat. In both cases cassava was planted in the mulch of weeds.

Except for *Arachis pintoi*, all cover crops germinated well and had established full soil cover at 3-4 months after planting. *Arachis pintoi* established more slowly. After harvest of the first cassava crop, all cover crops or weeds were slashed back and mulched, while a second crop of cassava was planted in manually prepared planting holes.

Table 9 shows the yields of CM507-37 for the two crop cycles, both in the fertilized and unfertilized experiments. In the check plots without cover crops or mulch,

fertilizer application nearly doubled cassava yields in the first, and tripled yields in the second year. Only cover cropping with *Macroptilium* increased cassava yields significantly in the second year in the absence of fertilizers, while all cover crops reduced yields in the presence of fertilizers. Yield reductions were most marked for *Desmodium ovalifolium* and *Arachis pintoi*, and were more serious in the second than in the first year of establishment. Fertilizer application stimulated the growth of forages, resulting in strong competition with cassava, mainly for soil water. Besides this strong competitive effect of the cover crops, it is possible that *Desmodium* and *Arachis* had an allelopathic effect (CIAT, 1993), as both cassava cultivars were seriously stunted in these treatments. MCol 1684 is less vigorous and has a less extensive root system than CM507-37 (CIAT, 1985). This resulted in lower yields and much greater depression due to the cover crops (CIAT, 1993). Thus, some cassava varieties are more suitable for cover cropping than others, but most cultivars will suffer from severe competition when associated with vigorously growing perennial forage legumes.

Table 9. Effect of various cover crops and weed mulch on the yield of cassava, cv. CM507-37, grown during two cropping cycles with and without fertilizer application in CIAT-Quilichao, Colombia, in 1987/88 and 1988/89.

	Fresh root yield (t/ha) 1987/88		Fresh root yield (t/ha) 1988/89	
Cover crop treatments	w/out fert.	with fert.	w/out fert.	with fert.
Sole cassava (no cover crop); weeds removed	29.6 bc	51.8 abc	17.1 c	56.2 ab
C + *Zornia latifolia* CIAT 728	22.7 cd	50.4 abc	19.7 bc	42.7 cd
C + *Desmodium ovalifolium* CIAT 13089	19.2 d	48.1 bcd	5.9 d	17.0 e
C + *Arachis pintoi* CIAT 17434	26.9 bcd	45.9 bcd	7.1 d	29.5 d
C + *Centrosema acutifolium* CIAT 5277	23.5 cd	44.1 cd	18.3 c	43.2 bc
C + *Pueraria phaseoloides*	30.9 bc	39.0 d	21.6 abc	35.1 cd
C + *Macroptilium atropurpureum* CIAT 535	26.7 bcd	40.9 cd	25.4 ab	32.5 cd
Sole cassava; weeds cut and mulched	39.6 a	60.9 a	21.9 abc	61.6 a
Sole cassava, weeds sprayed [1)] and mulched	33.8 ab	56.1 ab	27.0 a	45.3 bc
F-test:	fertilizer effect **		fertilizer effect **	
	cover crop effect **		cover crop effect **	
	fert. x cover crop *		fert. x cover crop **	

[1)] weeds sprayed with Paraquat

Table 9 also shows that cassava yields were significantly increased by mulching the native weeds, either by cutting the weeds or by spraying with Paraquat. Yields increased both in the absence and presence of chemical fertilizers. The weed mulch not only supplied nutrients to the crop, but also increased soil moisture and decreased the surface soil temperature (Cadavid *et al*., 1998). Thus, mulching of native weeds combined with minimum tillage (hand preparation of planting holes) produced much better results than intercropping with leguminous cover crops.

2. Cover cropping of cassava with forage legumes in Pluak Daeng, Thailand

After evaluating a large number of forage species for adaptation to soil and climatic conditions in Thailand, some species were identified as potential cover crops for use with cassava. These were tested in Pluak Daeng, Rayong province. Nine leguminous species were planted in double rows in between rows of cassava, cv. Rayong 1, spaced at 1.80 x 0.55 m. Cassava received 156 kg/ha of 15-15-15 fertilizers. All forage species established well, resulting in complete soil cover in 3-4 months after planting, except for *Arachis pintoi* and *Stylosanthes hamata*, which established more slowly. In the first year, cover crops were not cut back, resulting in competition with cassava, both for light and soil moisture during the dry season. After the first cassava harvest, all cover crops were slashed back and mulched. Plots were subdivided and cassava was replanted at a spacing of 1.10 x 0.90 m in 60-cm wide strips prepared either with a hand tractor or by spraying the cover crops with Paraquat. The same methodology was used in the third year. In the second and third year cover crops were regularly slashed back at 20 cm above the ground to reduce competition with cassava. Nevertheless, **Table 10** shows that cassava yields were low and severely affected by competition from the cover crops. Most competitive was *Stylosanthes guianensis*, followed by *Centrosema pubescens*. *Stylosanthes hamata* and *Arachis pintoi* were not very competitive during the first year of establishment, but became very competitive in subsequent years. Least competitive was *Centrosema acutifolium*, but this was partly due to less vigorous growth resulting in only partial soil cover (Tongglum *et al*., 1992)

Table 10. Effect of intercropping cassava with leguminous cover crops on the yield of cassava, cv. Rayong 1, during three consecutive years of cropping in Pluak Daeng, Thailand.

	DM cover crops (t/ha)		Cassava fresh root yield (t/ha)[1)]		
Cover crop treatments	1988/89[2)]	1990/91[3)]	1988/89	1989/90	1990/91
Sole cassava (no cover crop)	-	-	11.68 a	7.79 a	19.62 a
C + *Stylosanthes hamata*	1.74 d	1.68 ab	10.27 ab	3.91 c	4.45 de
C + *Stylosanthes guianensis*	9.22 a	2.19 a	3.21 d	6.56 ab	0.83 e
C + *Arachis pintoi*	0.87 d	-	8.46 bc	6.56 ab	9.71 cd
C + *Centrosema acutifolium*	2.17 bcd	0.93 bc	7.66 bc	6.69 ab	15.33 ab
C + *Centrosema pubescens*	1.04 d	1.34 bc	7.51 bc	5.60 bc	6.17 d
C + *Mimosa envisa*	1.97 cd	1.36 bc	7.49 bc	6.48 ab	13.33 bc
C + *Desmodium ovalifolium*	3.81 b	0.68 c	7.26 bc	6.78 ab	13.46 bc
C + *Macroptilium atropurpureum*	2.19 bcd	0.78 c	6.61 c	7.70 a	8.96 cd
C + *Indigofera* sp.	3.25 bc	1.27 bc	3.05 d	6.36 ab	8.50 c
F-test	**	**	**	*	**

[1)] Cassava received 25 kg N, 25 P_2O_5 and 25 K_2O/ha; data for 1989 and 1990 refer to those plots with tractor preparation of cassava planting strips

[2)] At 10 months after planting

[3)] At 3 months; average of mechanical and chemical land preparation treatments

A similar experiment was conducted in an adjacent field. In main plots two cassava plant spacings were used, i.e. 1.0 x 1.0 m and 1.50 x 0.67 m, both giving a plant population of 10,000 plants/ha. In subplots various forage species were planted in between

cassava rows. Cassava received 156 kg/ha of band applied 15-15-15 fertilizer. After the first cassava harvest, the cover crops were slashed back and cassava was replanted in 60-cm wide strips prepared with a hand tractor. In the second year all cover crops were well established and competed strongly with cassava, mainly for soil moisture during cassava establishment. **Table 11** shows that there were no significant differences in cassava yields due to plant spacing, but that nearly all cover crops reduced cassava yields, some more than 50%. Most competitive were *Indigofera* and *Mimosa envisa*, which were also among the most productive forage species tested. Less productive and thus less competitive were *Zornia glabra*, *Alysicarpus vaginales* and *Arachis pintoi*, although the latter still caused a marked yield reduction in the second year.

Table 11. Dry matter production of various cover crops and their effect on the yield of cassava, cv. Rayong 1, planted at either 1.0 x 1.0 m or at 1.5 x 0.67 m at Pluak Daeng, Thailand. Data are average values for the two plant spacings.

	DM cover crops (t/ha)		Cassava fresh root yield (t/ha)	
Cover crop treatments	1991/92	1992/93	1991/92	1992/93
Sole cassava (no cover crops)	-	-	18.61 a	7.14 a
C + *Indigofera* sp.	6.55	3.15	8.33 c	4.19 abc
C + *Zornia latifolium* CIAT 9199	1.08	1.14	16.34 ab	3.94 bc
C + *Zornia glabra* CIAT 8283	0.47	1.68	22.23 a	5.44 ab
C + *Alysicarpus vaginales*	1.37	0.27	17.19 ab	6.70 ab
C + *Mimosa envisa*	4.61	2.96	12.71 bc	2.15 c
C + *Stylosanthes hamata*	3.21	5.23	13.61 bc	2.12 c
C + *Arachis pintoi*	0.26	0.42	15.97 b	2.30 c
F-test for cassava yield: Cassava spacing (S):			NS	NS
Cover crops (C):			**	**
S x C:			NS	*

***Source**: Tonglum et al., 1992.*

From these three cover crop experiments it can be concluded that cassava is a weak competitor and yields are reduced markedly if the plants have to compete with deep rooted and well established forage legumes used as a cover crop. This competition is particularly strong during cassava plant establishment, especially when this coincides with a period of drought. Thus, cover cropping with most forage legumes would not be practical since it tends to reduce cassava yields and requires considerable additional labor. Ruppenthal (1995) also reported yield reductions of more than 40% when forage legumes were grown as cover crops under cassava in Quilichao and Mondomo, both in Cauca Department of Colombia. Ruppenthal *et al.* (1997) also showed that cover crops, once well established, were very effective in reducing soil erosion, but that erosion can be controlled more effectively and with less reduction of cassava yield with the use of contour hedgerows of vetiver grass (*Vetiveria zizanioides).*

Alley Cropping

Growing crops between hedgerows of leguminous tree species is called "alley cropping", and is another alternative to improve soil fertility and reduce soil erosion. The space between hedgerows can be varied, but is usually around 4-5 meters, so that less than 20% of the total land area is occupied by the hedgerows. The hedgerows are pruned before and at regular intervals after planting the crop and the prunings are distributed among crop plants to serve as a mulch, to supply nutrients (especially N), and to control weeds and erosion.

1. Adaptation of leguminous tree species to acid soils in Quilichao, Colombia

Eight leguminous tree species were evaluated on an acid soil (pH 4.13 with 79% Al saturation) in Quilichao to which four levels of lime had been applied, i.e. 0, 0.5, 2 and 6 t/ha of calcitic lime. Although no production data were taken, it was observed that *Cassia siamea* was by far the most productive under highly acidic soil conditions, followed by *Sesbania sesban*, *Clitoria fairchildiana* and *Gliricidia sepium*; in contrast, *Leucaena leucocephala* was most susceptible to soil acidity and only grew vigorously with application of 6 t/ha of lime.

2. Adaptation of leguminous shrub and tree species to conditions in Rayong, Thailand

Various leguminous shrubs were tested in Rayong, Thailand, to determine their general adaptation, ease of establishment, productivity of leaf/stem biomass, resistance to regular pruning and drought tolerance. **Table 12** shows that several species of *Sesbania* were highly productive in the first year, but did not resist regular pruning. Perennial pigeon pea varieties were easy to establish, were highly productive and drought tolerant, but they will last only a few years. *Leucaena leucocephala*, *Gliricidium sepium* and *Cassia siamea* were more difficult and slow to establish, but once established they were highly productive, resistant to pruning and very persistent. *Cassia siamea* is a non-N fixing legume tree and serves mainly to produce biomass as mulch, to recycle nutrients and protect the soil from erosion. Other species like *Flemingia congesta* and *Tephrosia candida* have been used successfully in other countries. Hedgerows consisting of a mixture of fast growing pigeon pea with a slower growing but more persistent tree specie like *Leucaena leucocephala* are being adopted by farmers in northern Thailand (Boonchee *et al.*, 1997).

3. Alley cropping of cassava with leguminous shrubs in Malang, Indonesia

The use of hedgerows of *Flemingia congesta* and *Gliricidia sepium* in cassava fields was investigated for four years in Malang, Indonesia. The experiment had eight treatments without replication. Eroded soil was collected in concrete channels below each plot. The two hedgerow species were initially difficult to establish and during the first three years they had no beneficial effect on cassava yield or erosion (Wargiono *et al.*, 1998). However, in the fourth year, when cassava in other plots suffered from severe N deficiency after intercropping with maize, the cassava plants in the alley-cropped treatments were tall and had dark green leaves, indicating that the prunings of the hedgerows had supplied considerable amounts of N.

Table 12. Total dry weight of prunings at three harvests as well as total nutrient content of the prunings of alley crop hedgerow species grown at Rayong Field Crops Research Center, Rayong, Thailand in 1990/91.

	Total dry matter (t/ha)					
	Months after planting			Total nutrient content (kg/ha)[1)]		
Alley crop hedgerow species	3	6	13.5	N	P	K
Leucaena leucocephala	0	0.55	11.97	-	-	-
Gliricidia sepium	0.10	0.02	0.68	19.81	1.63	28.19
Cassia siamea	0.18	1.22	25.40	525.69	37.25	668.12
Sesbania grandiflora	1.08	0.42	0.32	48.94	3.31	51.12
Sesbania sesban	2.97	2.52	0	79.00	8.12	115.56
Sesbania aculeata	4.81	1.31	0.39	130.12	12.37	125.75
Sesbania javanica	1.63	0.67	0.36	52.50	3.93	52.12
Sesbania rostrata	3.67	1.17	0	77.19	5.25	73.31
Pigeon pea from USA	2.30	3.69	14.99	388.25	26.37	480.12
Pigeon pea ICP 8094	3.74	2.68	12.44	345.43	22.62	403.00
Pigeon pea ICP 8860	3.63	4.55	14.64	383.75	28.19	527.06
Pigean pea ICP 11890	3.96	3.20	20.94	517.25	33.44	564.75

[1)] sum of nutrients in leaves and stems from three harvests

Table 13 indicates that during the fourth year the two alley-cropped treatments produced high cassava yields and the lowest levels of erosion (by enhanced early canopy cover). In a previous experiment at the same site, hedgerows of *Leucaena leucocephala* and *Gliricidium sepium* also produced the highest cassava yields and lowest levels of erosion during the fourth year of consecutive planting; these two treatments also resulted in the highest levels of soil OM, the lowest bulk density and the highest water infiltration rates and soil aggregate stability (Wargiono *et al.*, 1995). **Table 13** also shows that cover cropping with *Mimosa envisa* reduced cassava yields slightly in the first two years, but markedly in the subsequent two years. Thus, once well-established, hedgerows of leguminous shrubs significantly enhanced soil fertility and improved the soil's physical characteristics. However, in less fertile soils or in areas with a long dry season, the hedgerows can severely compete with neighboring cassava for water and nutrients (Jantawat *et al.*, 1994); they also require additional labor to keep properly pruned to prevent light competition.

Table 13. Effect of various crop/soil management practices on soil loss due to erosion and on cassava and maize yields during four consecutive cropping cycles on 5% slope in Jatikerto Experiment Station in Malang, Indonesia.

	Dry soil loss (t/ha)				Cassava root yield (t/ha)				Maize yield (t/ha)		
Crop/soil management treatments	91/92	92/93	93/94	94/95	91/92	92/93	93/94	94/95	91/92	92/93	93/94
C+M[1)], no fertilizers, no ridges	58.3	49.3	55.7	8.5	16.3	15.8	5.1	6.6	-	-	0
C+M[1)], no fertilizer, contour ridges	43.0	36.9	36.7	2.8	25.4	23.2	5.1	13.3	-	-	0
C+M, with fertilizer, contour ridges	39.2	24.8	28.1	3.8	20.4	20.5	17.8	16.7	1.98	2.27	2.88
C+M, with fert., contour ridges, elephant grass hedgerows	36.9	19.8	20.8	2.4	18.4	17.4	11.8	19.3	1.36	1.42	1.96
C+M, with fert., contour ridges, *Gliricidia* hedgerows	43.2	22.3	20.9	2.2	16.3	18.0	16.1	20.7	1.16	1.28	2.80
C+M, with fert., contour ridges, *Flemingia* hedgerows	41.3	17.7	17.3	1.0	17.2	18.1	14.2	21.6	1.26	1.46	3.20
C+M, with fert., contour ridges, *Mimosa* cover crop	38.4	18.3	24.7	2.4	17.1	18.2	12.2	9.9	1.44	1.63	3.36
C+M[1)], with fert., contour ridges, peanut intercrop	36.4	21.7	26.3	4.5	23.7	23.7	19.9	25.3	-	-	2.10

[1)] During the first two years there was no intercropped maize in treatments 1, 2 and 8; C+M = cassava intercropped with maize.

Intercropping

Intercropping cassava with short-duration crops is a common practice among smallholder farmers in many tropical countries. These intercrops are useful because they supply either food or additional income, especially at times when the cassava crop can not yet be harvested; they may fix N and supply other nutrients to the topsoil; they may protect the soil from the direct impact of rainfall when the cassava canopy is not yet closed, thus reducing soil erosion; and they may reduce weed growth during the early stages of cassava development. However, intercrops need to be carefully managed in order to reduce the competition with cassava, for light, water and nutrients. This will be discussed in more detail in Chapter 23**.** Only one example of the long-term effect of intercrops and alley crops on soil fertility and net income is shown below.

Long-term effect of intercropping, green manuring and alley cropping on cassava yield, net income and soil fertility

A long-term experiment was initiated in 1992 at Hung Loc Agric. Research Center in South Vietnam to determine the best cropping system to maintain high cassava yields and/or improve soil fertility. The eight treatments included cassava monoculture, two intercropping, three green manure and two alley cropping systems, as indicated in **Tables 14** and **15.** During the first seven years all plots received a uniform fertilizer application which obscured the effect of the various cropping systems; in years 8, 9 and 10 no chemical fertilizers were applied, which resulted in a significant drop in cassava yields. As of the 11^{th} crop all plots were split, with half being fertilized every year and half remaining unfertilized. Cassava, cv. KM 60 was planted every year at a spacing of 1.0 x 1.0 meter, and the various intercrops and green manures were planted at the same time and in between cassava rows. For the two alley cropping treatments, each fifth row of cassava was replaced by one row of the hedgerow species; these were planted from seed only in the first year. The hedgerows were pruned every year before planting cassava and the prunings were mulched between the four cassava rows nearby. Soil samples were taken nearly every year after land preparation and before the next cassava planting. **Table 14** shows the results of soil analyses after the third and after the 15th year of continuous cassava cropping. Fifteen years of continuous cassava cropping had decreased soil pH in all treatments, but especially when no fertilizers were applied and in both alley cropping treatments. There was also a significant reduction in the level of soil OM, but less so when fertilizers had been applied and in the two alley cropping treatments. Yearly application of 80 kg N, 40 P_2O_5 and 80 K_2O/ha generally increased the levels of available P, had little effect on Ca and Mg, but actually decreased the level of exchangeable K, probably due to increased K removal with the higher root yields obtained. While most intercrop and green manure treatments had little effect on soil fertility characteristics, the soil in the two alley cropping treatments had markedly lower pH, and higher levels of OM, P, Ca, Mg and K. Thus, of the various biological soil improvement treatments, alley cropping was the only system that actually had a long-term beneficial effect on soil fertility, although not quite enough to maintain the original soil fertility characteristics after 15 years of continuous cassava cropping , even in combination with some fertilizers.

Table 14. Effect of planting intercrops, green manures and alley crops, with or without fertilizers, on soil fertility characteristics after 15 years of continuous cassava cultivation at Hung Loc Agric. Research Center in Dongnai, Vietnam in 2007/08. (before 16th crop planting)

	pH		OM(%)		P(ppm)		Al(me/100g)		Ca(me/100g)		Mg(me/100g)		K(me/100g)	
Treatments[1)]	-fert	+fert	-fert	+fert	-fert	+fert	-fert	+fert	-fert	+fert	-fert	+fert	-fert	+fert
3rd year (1994)	4.4		3.1		9.4		1.00		1.66		0.57		0.32	
16th year (2007)														
1. C monoculture[1)]	4.11	4.38	2.24	2.35	15.73	13.96	2.80	2.91	0.63	0.64	0.16	0.15	0.16	0.13
2. C+pigeon pea GM	4.09	4.43	2.46	2.52	14.53	16.31	2.96	2.81	0.58	0.63	0.15	0.14	0.13	0.15
3. C+*Mucuna* GM	4.12	4.34	2.35	2.46	14.33	13.08	2.81	2.76	0.72	0.66	0.22	0.17	0.14	0.10
4. C+peanut IC	4.06	4.35	2.48	2.59	18.86	26.39	3.07	2.86	0.55	0.71	0.16	0.14	0.13	0.12
5. C+cowpea IC	4.11	4.28	2.36	2.07	17.70	19.23	2.91	2.70	0.49	0.67	0.17	0.14	0.22	0.13
6. C+*Crotalaria* GM	4.14	4.30	2.44	2.56	15.00	16.26	2.81	2.76	0.66	0.62	0.15	0.16	0.16	0.13
7. C+*Leucaena* AC	3.97	4.21	2.82	3.08	18.26	28.82	2.86	2.55	0.76	0.82	0.25	0.26	0.23	0.18
8. C+*Gliricidia* AC	3.98	4.20	2.51	2.62	15.33	21.77	2.86	2.76	0.78	0.63	0.25	0.17	0.22	0.14
Average	**4.07**	**4.31**	**2.46**	**2.53**	**16.22**	**19.47**	**2.74**	**2.76**	**0.65**	**0.67**	**0.19**	**0.17**	**0.17**	**0.14**

[1)] Cassava variety is KM 60; -F = without fertilizers; +F = with 80 kg N, 40 P_2O_5, 80 K_2O/ha
GM = green manure, IC = intercrop, AC = alley crop

Table 15 shows the effect of the various treatments on the yield of cassava, the root starch content and the gross and net income during the 16th year of cropping. Highest cassava yields, starch contents and gross and net income were obtained with the two alley cropping treatments, with hedgerows of *Leucaena leucocephala* usually being more effective than *Gliricidia sepium*, in spite of the very low soil pH. The beneficial effect of the two alley cropping treatments became only apparent during the 8th cropping cycle, but has been consistent ever since, most markedly in the unfertilized treatments. Among the two intercrops, peanut was better than cowpea, while the three green manures only had a beneficial effect on cassava yields in the absence of chemical fertilizers (CIAT, 2008).

From these various experiments mentioned above, and many more reported in the literature, it can be concluded that cassava is a very weak competitor and suffers serious setbacks if it has to compete with weeds, intercrops or cover crops, especially at the early stage of establishment due to its slow initial rate of growth. Thus, most perennial cover crops will strongly compete with cassava at the early stages of growth resulting in low cassava yields. Most intercropped green manures or long-duration intercrops will also tend to reduce cassava yields. Most beneficial are some of the green manures when they are grown and incorporated before planting cassava, but only in areas with a long wet season that provides sufficient soil moisture during most of the cassava growth cycle; their beneficial effect is most pronounced when no chemical fertilizers are applied.

Among the various biological solutions mentioned above, alley cropping seems to have the greatest long-term beneficial effect on cassava yields and soil fertility, but more so in the absence than in the presence of chemical fertilizers. Once established the hedgerows require little maintenance besides regular pruning and they can survive for at least 15-20 years without the need for replanting. Besides improving soil fertility, the prunings when mulched on the soil surface, will also help to control weeds and erosion, reduce soil surface

temperatures and increase soil moisture. Similar beneficial effects of mulching have also been obtained when native weeds were cut and mulched before planting cassava with minimum tillage.

Table 15. Effect of planting intercrops, green manures and alley crops, with or without fertilizers, on cassava and intercrop yields, as well as the gross and net income obtained when cassava, KM 60, was grown for the 16th consecutive year at Hung Loc Agric. Research Center in Dongnai, Vietnam in 2007/08.

	Root yield (t/ha)		Starch content (%)		Gross income[2] ('000 d/ha)		Product. costs[3] ('000 d/ha)		Net income ('000 d/ha)	
Treatments[1]	+fert	-fert	+fert	-fert	+fert	-fert	+fert	-fert	+fert	-fert
C monoculture	17.44	4.81	23.28	21.28	20,405	5,628	6,008	3,800	14,397	1,828
C+pigeon pea GM	15.62	6.75	23.60	21.70	18,275	7,898	8,108	5,900	10,167	1,998
C+*Mucuna* GM	17.82	8.56	24.45	22.35	20,849	10,015	8,108	5,900	12,741	4,115
C+peanut IC[4]	20.41	8.62	25.35	24.08	24,824	10,085	8,108	5,900	16,716	4,185
C+cowpea IC	19.44	7.44	24.92	22.65	22,745	8,705	8,108	5,900	14,637	2,805
C+*Crotalaria* GM	18.75	8.50	24.95	21.72	21,938	9,945	8,108	5,900	13,830	4,045
C+*Leucaena* AC	20.68	13.39	25.52	24.40	24,196	15,666	7,708	5,500	16,488	10,166
C+*Gliricidia* AC	19.30	16.75	26.32	24.95	22,581	19,597	7,708	5,500	14,873	14,097
Average	**18.68**	**9.35**	**24.80**	**22.89**	**21,977**	**10,942**	**7,745**	**5,538**	**14,231**	**5,404**

[1] C = cassava, GM = green manure, IC = intrcrop, AC = alley crop

[2] Prices: cassava dong 1,170 /kg fresh roots; peanut 8,000/ dry pods

[3] Costs

land preparation	900,000/ha	cassava planting	700,000/ha
fertilizers (80:40:80 kg/ha)	1,983,000/ha	weeding	2,200,000/ha
-urea (46% N)	5,500/kg	intercrop planting	500,000/ha
-SSP (17% P_2O_5)	1,700/kg	intercrop harvest	1,200,000/ha
-KCl (60% K_2O)	4,700/kg	seed of intercrops or GM	400,000/ha
fertilizer appl. (5 mandays/ha)	225,000/ha	cost of labor	45,000/manday

[4] Peanut yield with fertilizers: 118 kg dry pods; without fertilizers: 0 yield

[5] 1 US$ = 17,000 dong in 2008

Source: Nguyen Huu Hy, personal communication.

Application of Animal Manures and Compost

Farmers who have no access to, or can not afford to buy chemical fertilizers often try to maintain the fertility of their soil by the application of animal manures, while others prefer to apply a combination of manures or compost and small amounts of chemical fertilizers.

Animal manures and compost have the advantage that they may be available and free on the farm. They supply not only all the essential plant nutrients, including secondary and micro-nutrients, but also organic matter which will stimulate micro-organisms in the soil, improve soil structure, aggregate stability, and water and nutrient-holding capacity. The disadvantage of animal manures is that they are bulky, having high water and low nutrient contents; this makes transport and handling expensive or cumbersome, especially in mountainous areas. **Table 16** shows that a 50 kg bag of 15-15-15 chemical fertilizer contains about the same amount of the major nutrients as one ton of manure or compost.

Table 16. Average nutrient content of one ton of various types of wet manure and compost as compared to 50 kg of 15-15-15 chemical fertilizers.

		kg		
	% DM	N	P	K
1 t cattle manure	32	5.9	2.6	5.4
1 t pig manure	40	8.2	5.5	5.5
1 t chicken manure	57	16.6	7.8	8.8
1 t sheep manure	35	10.5	2.2	9.4
1 t city garbage compost	71	6.9	3.3	6.1
50 kg 15-15-15 fertilizer	100	7.5	3.3	6.2

Source: *Howeler, 2001.*

Another problem is that both moisture and nutrient contents are highly variable, as shown in **Table 17**. This makes it difficult to know how much to apply and impossible to tailor the application to the specific requirements of the soil and crop.

Several experiments have been conducted to determine the effectiveness of various manures in comparison with chemical fertilizers in increasing cassava yields.

1. The use of pig manure and chemical fertilizers in Vietnam

An experiment was conducted at Thai Nguyen University in Thai Nguyen, North Vietnam, to compare the effectiveness of various rates of pig manure with chemical fertilizers, or a combination of manure and fertilizers in increasing cassava yields and net income. **Table 18** shows that cassava yields increased from 3.25 to 13.11 t/ha with the application of 15 t/ha of wet pig manure. However, yields of 15.47 t/ha were obtained with the application of 80 kg N and 80 K_2O/ha, while yields of 18.70 t/ha were obtained with the combination of 80 kg N, 80 kg K_2O and 10 ton of pig manure/ha. Considering the cost of fertilizers and the cost of manure application, the highest net income was obtained with the combined application of the chemical fertilizer with 5 t/ha of pig manure.

2. The use of cattle manure, compost and chemical fertilizers in Indonesia

A similar experiment was conducted in Jatikerto Experiment Station near Malang, Indonesia to compare the effectiveness of cattle manure or compost with various combinations of N, P and K fertilizers, applied either alone or in combination with manure or compost, in increasing the yields of cassava and intercropped maize as well as net income. **Table 19** shows that cassava yields increased from 10.96 to 37.47 t/ha, while the intercropped maize yields increased from 1.10 to 2.10 t/ha with the application of 135 kg N, 50 P_2O_5 and 100 K_2O/ha, while cassava yields were only 26.53 and 22.67 t/ha with the application of 10 t/ha of cattle manure and compost, respectively. Highest cassava yields and net income were obtained with the combination of 135 kg N/ha and 5 t/ha of compost.

Table 17. Nutrient content of animal manures and composts, as reported in the literature.

Source of manure/compost	% Moisture	(% of dry material) C	N	P	K	Ca	Mg	S
Buffalo manure[1)]	60.4	17.4	0.97	0.58	1.28	-	-	-
Dairy cattle manure[2)]	79.0	-	2.66	0.48	2.38	1.33	0.52	0.23
Fattening cattle manure[2)]	80.0	-	3.50	1.00	2.25	0.60	0.50	0.43
Cattle manure[1)]	46.4	16.9	1.11	0.44	1.56	-	-	-
Cattle manure[3)]	-	-	2.00	0.65	1.67	2.86	0.60	0.20
Cattle manure (Dampit, Indonesia)[4)]	-	-	1.43	2.96	1.60	2.13	0.96	-
Cattle manure (Indonesia)[5)]	-	39.1	1.87	0.56	1.09	0.57	0.23	-
Cattle manure (Costa Rica) [6)]	-	-	2.23	0.77	2.25	1.77	0.89	-
Cattle manure[8)]	75.0	-	2.40	0.61	2.67	-	-	-
Cattle manure[9)]	-	-	0.35	0.06	0.16	-	-	-
Average cattle manure	**68.2**	**-**	**1.85**	**0.81**	**1.69**	**1.54**	**0.62**	**0.29**
Pig manure[1)]	29.9	19.0	1.32	2.37	0.96	-	-	-
Pig manure[2)]	75.0	-	2.00	0.56	1.52	2.28	0.32	0.54
Pig manure[8)]	75.0	-	2.80	1.22	1.67	-	-	-
Average pig manure	**60.0**	**-**	**2.04**	**1.38**	**1.38**	**-**	**-**	**-**
Chicken manure[3)]	-	-	5.00	1.31	1.25	2.86	0.60	0.80
Chicken manure (Blitar, Indonesia)[4)]	-	-	1.75	0.23	0.77	6.82	1.46	-
Chicken manure (Blitar, Indonesia)[4)]	-	-	0.43	0.67	0.39	4.93	1.43	-
Chicken manure (Khaw Hin Sorn, Thailand)[4)]	-	-	1.25	0.43	1.27	1.31	0.37	-
Chicken manure (Costa Rica)[6)]	-	-	1.68	2.58	1.19	6.90	0.66	-
Chicken manure (Pescador, Colombia)[7)]	-	-	4.96	1.95	2.27	4.53	0.48	-
Chicken manure (layer)[8)]	70	-	5.00	1.89	2.50	-	-	-
Chicken manure (broiler)[8)]	40	-	4.83	1.82	2.50	-	-	-
Chicken dropping[9)]	-	-	2.80	1.33	1.04	-	-	-
Chicken manure[9)]	-	-	2.87	1.27	1.83	-	-	-
Broiler chicken manure[10)]	25.0	-	2.26	1.08	1.67	-	-	-
Hen manure[10)]	37.0	-	2.06	1.90	1.81	-	-	-
Average chicken manure	**43.0**	**-**	**2.91**	**1.37**	**1.54**	**4.56**	**0.83**	**-**
Horse manure[2)]	60.0	-	1.72	0.25	1.50	1.96	0.35	0.17
Duck manure[1)]	22.2	21.4	1.02	1.38	0.90	-	-	-
Sheep manure[3)]	-	-	2.00	0.65	2.50	1.78	1.20	0.60
Sheep manure[2)]	65.0	-	4.00	0.60	2.86	1.67	0.53	0.26
Average sheep manure	**-**	**-**	**3.00**	**0.62**	**2.68**	**1.72**	**0.86**	**0.43**
Human manure[9)]	-	-	1.20	0.06	0.21	-	-	-
City garbage compost (Bangkok)[1)]	28.8	17.3	0.97	0.46	0.86	-	-	-
City compost[9)]	-	-	1.75	0.44	1.25	-	-	-
Rural compost[9)]	-	-	0.75	0.20	0.60	-	-	-
Average city/rural compost			**1.16**	**0.37**	**0.90**	**-**	**-**	**-**

Table 17. (continued)

Source of manure/compost	% Moisture	(% of dry material) C	N	P	K	Ca	Mg	S
Rice straw compost[1)]	73.7	33.8	1.07	0.19	0.69	-	-	-
Rice straw[9)]	-	-	0.40	0.10	0.40	-	-	-
Rice husk[9)]	-	-	0.62	0.08	1.25	-	-	-
Peanut stem + leaves compost[1)]	58.6	11.6	0.81	0.10	0.38	-	-	-
Water hyacinth[1)]	-	-	2.00	1.00	2.30	-	-	-
Ash (rice husks)[4)]	-	-	0.03	0.40	1.06	0.47	0.22	-
Fly ash (Nanning, China)[4)]	-	-	0.09	<0.10	1.20	4.14	1.14	-
Wood ash (Trivandrum, India)[11)]	-	-	-	-	8.70	20.8	1.90	-
Wood ash[3)]	-	-	-	0.87	4.17	23.2	2.10	0.40

[1)] Suzuki *et al.*, 1988
[2)] Loehr, 1968
[3)] Jacob and Uexkull, 1973
[4)] Howeler (unpublished)
[5)] Sutanto *et al.*, 1993
[6)] Don Kass (personal communication)
[7)] Amezquita *et al.*, 1998
[8)] Scaife and Bar-Yosef, 1995
[9)] FADINAP
[10)] Perkins *et al.*, 1964
[11)] Kabeerathumma *et al.*, 1990

Table 18. Effect of the application of FYM[1)] and chemical fertilizers on cassava yield and economic benefit at Thai Nguyen University of Agric. and Forestry inThai Nguyen province of Vietnam, in 2001 (2nd year).

Treatments[1)]	Cassava root yield (t/ha)	Height at 8 months (cm)	Leaf life at 3 months (days)	HI	Gross income[2)]	Fert. costs[2)]	Product. costs[3)]	Net income
					----------('000 dong/ha)----------			
1. no fertilizers, no FYM	3.25	87.1	46.5	0.39	1,625	0	2,800	-1.175
2. 5 t FYM/ha	7.79	116.6	55.2	0.49	3,895	500	3,300	0.595
3. 10 t FYM/ha	10.02	133.9	65.0	0.52	5,010	1,000	3,800	1.210
4. 15 t FYM/ha	13.11	151.8	66.1	0.52	6,555	1,500	4,300	2.255
5. 80 N+80 K_2O/ha, no FYM	15.47	154.5	66.8	0.50	7,735	680	3,580	4.155
6. 80 N+80 K_2O/ha + 5 t FYM/ha	17.98	180.0	68.5	0.48	8,990	1,180	4,080	4.910
7. 80 N+80 K_2O/ha + 10 t FYM/ha	18.70	188.3	70.8	0.49	9,350	1,680	4,580	4.770
8. 80 N+80 K_2O/ha + 15 t FYM/ha	18.50	196.6	73.1	0.48	9,250	2,180	5,080	4.170

[1)] FYM = farm yard manure (pig manure)
[2)] Prices: cassava dong 500/kg fresh roots
urea (45% N) 2,100/kg
KCl (60% K_2O) 2,300/kg
manure+application 100/kg
[3)] Cost of cassava cultivation: 2.8 mil. dong/ha
Cost of chemical fertilizer application 0.10 mil. dong/ha
Source: *Nguyen The Dang, personal communication, 2002.*

Table 19. Effect of various fertilization alternatives on the yields of cassava, cv Faroka, and intercropped maize as well as gross and net income when grown in Jatikerto Station in Malang, East Java, Indonesia, in 2005/06 (2nd year).

Treatments N-P_2O-K_2O (kg/ha)	Organic (t/ha)	Maize yield[2] (t/ha)	Cassava yield (t/ha)	Gross income[3]	Fertil. costs[3]	Prod. costs[4]	Net income	Farmers preference ranking
				(mil. Rp/ha)				
1. 0-0-0	0	1.10	10.96	4.72	0	4.10	0.62	
2. 135-0-0	0	1.93	35.60	13.52	0.45	7.01	6.51	2
3. 135-50-0	0	2.07	36.80	14.05	0.69	7.37	6.68	3
4. 135-50-100	0	2.10	37.47	14.30	1.27	8.02	6.28	4
5. 0-0-0	10 cattle manure	1.66	26.53	10.32	2.00	7.65	2.67	
6. 0-0-0	10 compost	1.63	22.67	9.05	1.00	6.27	2.78	
7. 135-0-0	5 cattle manure	2.26	35.63	13.89	1.45	8.01	5.88	1
8. 135-0-0	5 compost	1.97	39.33	14.75	0.95	7.88	6.87	5
9. 135-50-0	5 compost	1.87	39.07	14.56	1.19	8.10	6.46	
10. 135-0-0	5 sugar mud[1]	1.67	33.73	12.63	0.95	7.32	5.31	

[1] sugar mud = blotong = by-product of sugar mill

[2] maize grain yield

[3] Prices:

cassava:	Rp 320/kg fresh roots	KCl (60% K_2O)	Rp 3,500/kg
maize	1,100/kg dry grain	cow manure	200/kg
urea (45% N)	1,500/kg	compost	100/kg
SP-36 (36% P_2O_5))	1,700/kg	sugar mud	100/kg

[4] Costs: cassava harvest+transport 100/kg

production costs, without fertilizers or cassava harvest, estimated at Rp 3 mil/ha

1 US$ is about 9,000 ruphias

Source: *Utomo et al., 2010.*

From these experiments it may be concluded that the application of the right amount and balance of N, P and K in chemical fertilizers tends to be more effective in increasing cassava (and intercrop) yields than the application of animal manures or compost, even at fairly high rates of application of the latter. But these and other experiments have also shown that the combination of medium levels of manure or compost with the right balance of N, P and K in chemical fertilizers will produce the highest yields and net income. In this case the chemical fertilizers will supply most of the major nutrients that are needed for a particular soil and crop, while the manure supplies some additional nutrients as well as organic matter to improve the physical conditions of the soil. Similarly, the combination of chemical fertilizers with alley cropping, intercropping, green manuring or the application of mulch will generally give the highest yields and income.

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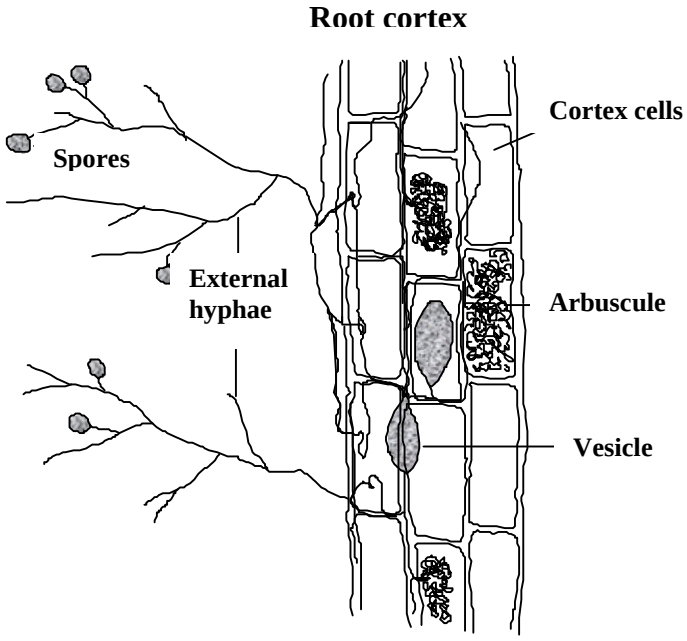
CHAPTER 19

IMPORTANCE OF MYCORRHIZA FOR PHOSPHORUS ABSORPTION BY CASSAVA [1]

Reinhardt Howeler[2]

Cassava is generally known as a crop that will grow well in very acid and infertile soils, where many other food crops would perish. One reason why cassava grows better in low-fertility soils then most other crops is that it is very tolerant of low levels of available P in the soil. As indicated in Chapter 16, this is not because cassava has a better root system or has a more efficient P absorption capacity. In fact, cassava has a very coarse and poorly branched root system, which is inefficient in exploring a large volume of surrounding soil for nutrient extraction. However, in practically all natural soils, the fibrous roots of cassava soon become infected with vesicular-arbuscular (VA) mycorrhizal fungi, which produce vesicles and arbuscules in the cortex of the fibrous roots, from which grow internal and external hyphae. These hyphae in turn produce spores, which can survive for long periods of time in the soil without the presence of plant roots. Once plant roots grow near the spores, the latter will germinate and infect the roots by producing vesicles and arbuscules in the root cortex (**Figure 1**).



[1] For color photos see pages 768-770.

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These VA-mycorrhizal fungi (VAM) are present in nearly all natural soils and they infect the roots of the great majority of plants, including the major food crops. In this symbiotic association, the fungus utilizes carbohydrates produced by the plant, while the plant benefits from the increased uptake of P and some other nutrients through the external hyphae

Figure 1. Morphological structures of vesicular-arbuscular (VA) mycorrhiza.
Source: *adapted from Sieverding, 1984*

fect of myco root system, ow-mobility in the soil solution, such as P, Zn and Cu. While non-mycorrhizal roots absorb P mainly through the root hairs, which may extend 1-2 mm from the root surface, the mycorrhiza-infected roots absorb P mainly through the external hyphae, which may extend several centimeters into the soil. As such, these roots can explore a much larger volume of soil from which to absorb P and other low mobility nutrients, as shown in **Figure 2.**

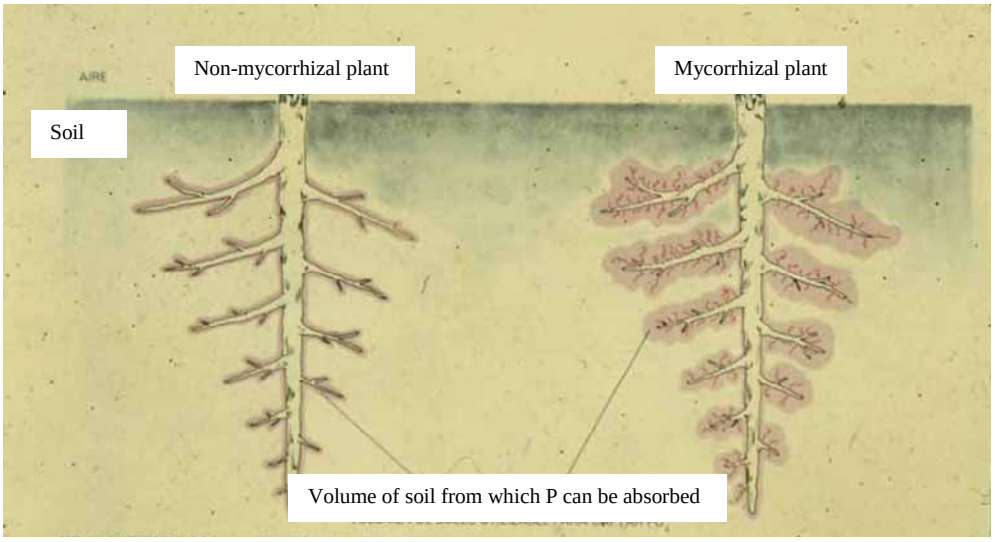

Figure 2. Schematic diagram of roots without (left) and with infection by mycorrhizal fungi, and the effect on the volume of soil from which P can be absorbed.

Among the tropical root and tuber crops, cassava and yam can grow well in soils that are extremely low in P; taro and sweet potato require intermediate levels; and Irish potato can produce well only at very high external P concentrations, as shown in Table 4 of Chapter 16. The cereal grains and grain legumes have intermediate P requirements, but tomato, Chinese cabbage and lettuce have P requirements as high as that of potato. However, when cassava and several other crops were grown in flowing nutrient solution culture, the external P requirements of cassava was one to two magnitudes higher than those of other crops tested. This anomalously high P requirement of cassava in nutrient solutions is due to its coarse and inefficient root system (Howeler *et al.*, 1982a) and the absence of mycorrhiza in most nutrient solutions. In normal soils, however, cassava roots are almost always infected with VA mycorrhizal fungi, which help the plant absorb soil P more efficiently than most other crops. That explains cassava's low P requirement in soil solution and low critical soil P level (see Table 4 in Chapter 16). When VA mycorrhizal fungi were eliminated from natural soil by sterilization with methyl bromide, Vander Zaag

et al. (1979) found that cassava growth was seriously reduced and the P concentration of leaves was reduced from 0.30 to 0.11%, indicating the important role of mycorrhiza in P uptake from low-P soils. Similar effects of soil sterilization on growth and P uptake by cassava were later reported by Howeler and Sieverding (1983) and by Howeler *et al.* (1982b, 1982c, 1987).

1. Cassava response to mycorrhizal inoculation in flowing nutrient solution culture

Further evidence of the role of mycorrhiza in P uptake was provided by the fact that when eight cassava cultivars were inoculated with VA mycorrhizal fungi in flowing nutrient solutions with an intermediate P concentration of 1 µM, both plant growth and the P concentration of tops and roots markedly improved (**Table 1**) (Howeler *et al.*, 1981; 1982a). The advantage of a flowing nutrient solution culture is that plants are grown in very large volumes of solution, in which the concentration of nutrients can be carefully controlled and maintained at a constant, and often very low, level, similar to the concentrations found in a buffered soil solution environment.

Table 1. Effect of the P concentration in solution and VA-mycorrhizal inoculation on the average percent VAM infection in roots, total dry matter production, and the P concentration of plant tops and roots of eight cassava cultivars grown in flowing nutrient solution at the University of Queensland, Australia.

P in solution	Root infection (%)		Total DM (g/plant)		P in tops (%)		P in roots (%)	
(µM)	Non-inoc.	Inoc.	Non-inoc.	Inoc.	Non-inoc.	Inoc.	Non-inoc.	Inoc.
0.1	nil	30	2.24	2.15	0.071	0.087	0.094	0.139
1	nil	38	3.72	5.55	0.168	0.214	0.122	0.401
10	nil	nil	9.94	9.04	0.351	0.339	0.368	0.412
100	nil	nil	9.10	8.48	0.494	0.457	0.595	0.503

***Source**: Howeler, 1980.*

At the lower concentration of 0.1 µM P, plants were extremely stunted with typical symptoms of P deficiency. Inoculation with VA-mycorrhiza at this very low P concentration did not improve plant growth but did reduce the severity of the deficiency symptoms. At the two highest concentrations of 10 and 100 µM P, plants grew vigorously irrespective of inoculation treatments. At the intermediate level of 1 µM P, plant growth was only slightly better than at 0.1 µM P during the first three weeks. However, during the last three weeks the inoculated plants improved considerably showing no more deficiency symptoms, while the non-inoculated plants remained extremely P deficient. In contrast, maize, rice, cowpea and beans were stunted and P deficient only at the lowest concentration of 0.1 µM P and reached maximum growth at the next level of 1 µM P. No beneficial effect of inoculation was observed in any of these species, which all have a rather fine and extensively branched root system.

Careful observation of the root system of cassava plants revealed that those of inoculated plants at the two lowest P concentrations were covered with a slimy substance, especially near the solution surface. Microscopic examination and staining with trypan blue, according to the method of Phillips and Hayman (1970), revealed that this substance consisted of masses of mycorrhizal hyphae covering the root surfaces and forming an intensive network of mycelium between the roots. Inside the roots these hyphae were

connected to vesicles (**Photo 1**). At the two highest P concentrations and in all of the non-inoculated treatments the roots were free of slime and no vesicles or hyphae were observed; roots of all the other plant species were free of slime as well as mycorrhizal infection in all treatments.

Cassava roots of inoculated plants at 0.1 µM P were clearly infected with mycorrhiza, which resulted in a significant increase in the P concentration of both tops and roots, but concentrations were still too low to cause a significant increase in plant growth and DM production. At 1 µM, however, inoculation increased the P concentration of tops from 0.17 to 0.21% and of roots from 0.12 to 0.40% and resulted in a DM increase of about 50% (**Photo 2**). Increases in DM production due to inoculation varied among cultivars from 16 to 103%, indicating that cultivars differ significantly in their response to mycorrhizal infection. At 10 and 100 µM P, cassava produced maximum yields and had a P concentration in the tops near or above the critical level of 0.4% (Howeler, 1978). At these high concentrations inoculation had no beneficial effect, either in terms of tissue P concentrations or DM production. At the intermediate P concentration of 1 µM, root growth of non-inoculated cassava plants was very poor, but when inoculated it improved considerably, resulting in a great number of fine roots. Thus, it appears that without mycorrhizal infection cassava has a very coarse and inefficient root system, which explains its high external P requirement in non-mycorrhizal nutrient solutions, whereas inoculation greatly improved P uptake, resulting in a more vigorous plant and a more effective root system. This would allow mycorrhizal cassava to absorb P even from very low-P soils.

Once it became clear that cassava is highly dependent on an effective mycorrhizal association for the uptake of P and possibly other nutrients, many experiments were initiated, first pot experiments in the greenhouse and later in the field, with the objective of studying ways to benefit from this association, to determine the specific characteristics of certain mycorrhizal species, differences among cassava varieties and the interaction with environmental conditions such as the pH and nutrient status of the soil, soil temperature and moisture and the effect of certain chemicals that may interfere with the effective functioning of the symbiosis.

2. Cassava response to mycorrhizal inoculation in pot experiments in the greenhouse

A detailed pot experiment was conducted at the Univ. of Queensland to study the interaction between VA mycorrhiza and the P status of the soil, and their effect on the growth and dry matter production of cassava. The experiment was conducted using a highly P-deficient and P-fixing soil to which eight levels of P had been applied, ranging from 0 to 16 t/ha of P in the form of $Ca(H_2PO_4)_2.H_2O$. In half of the pots the soil was sterilized with methyl bromide to kill all the native VAM. Cassava tip cuttings of cv. MAus 10 were rooted in small peat pots in a misting chamber; half of these cuttings were inoculated with 2-3 g of mycorrhiza-infected cassava roots, while the other half received the same amount of dead inoculum, which had been sterilized previously with methyl bromide. Once the roots of the tip cuttings had penetrated the walls of the peat pots, these pots with plants were transplanted into the test soil with the various P levels applied. The P concentration in the soil solution varied from less than 1 µM in the check to 700 µM P in the soil that had received the equivalent of 16 t/ha of P.

After about two weeks, plants started to show a response to applied P. In the sterilized soil at low P levels, plants showed typical symptoms of extreme P deficiency and started to lag behind those in the unsterilized soil. At 4-5 weeks a positive response to inoculation was observed and at six weeks this response was very marked and consistent at intermediate P levels, especially in the sterilized soil. At two months, plants were harvested and the fibrous roots stained for observation of mycorrhizal infection. **Figure 3** shows the effect of P application, sterilization and inoculation on total dry matter (DM) production.

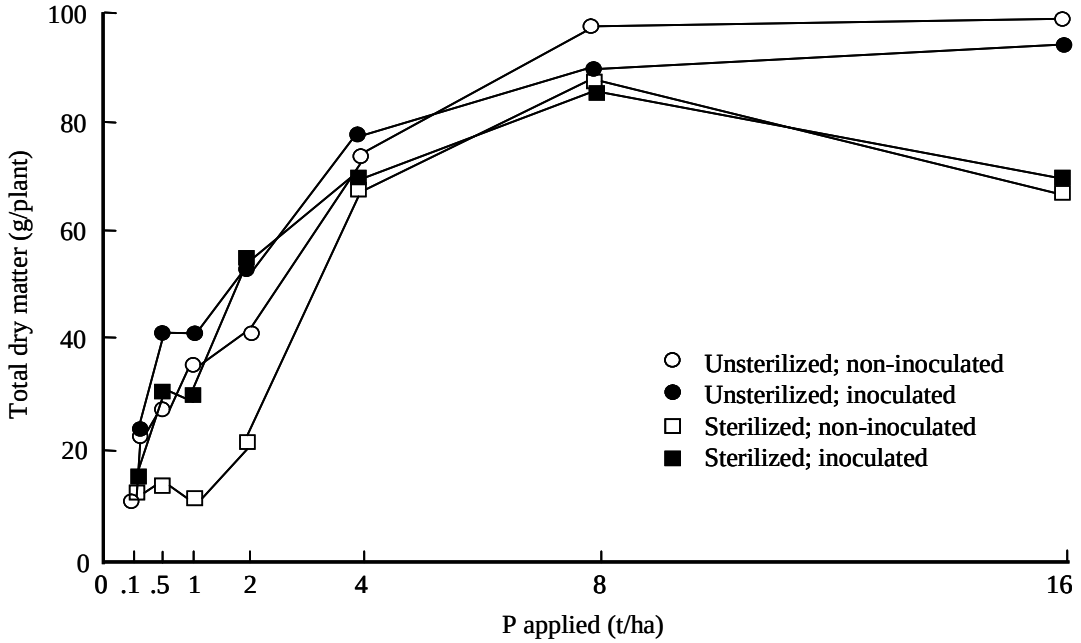

Figure 3. Effect of soil sterilization, mycorrhizal inoculation and P application on total dry matter production of cassava, cv. MAus 10, grown for two months in an Oxisol in the greenhouse at the University of Queensland, Australia.
***Source**: Howeler, 1980.*

Maximum DM production was reached at 8 t P/ha, irrespective of mycorrhizal treatments. In the sterilized soil higher P rates depressed yield due to salinity, which apparently resulted from a combination of extremely high P levels and a methyl-bromide induced increase in the inorganic N concentrations of the soil solution (Yost and Fox, 1979; Rovira, 1967; Lopez and Wollum, 1976). In the unsterilized soil inoculation increased DM production only at the intermediate P levels of 0.5, 1 and 2 t/ha. In the sterilized soil, however, inoculation increased plant growth up to 4 t P/ha., whereas at 2 t P/ha DM production increased as much as 3-fold. The beneficial effect was even more pronounced in terms of total P uptake by the plant, which increased more than 7-fold at 2 t/ha applied P in the sterilized soil. Inoculation also increased the tissue concentration as well as the total uptake of Ca and Mg, and increased the total uptake of K and Zn (Howeler *et al.*, 1982c). It is uncertain, however, whether this is a direct effect on the uptake of these elements or whether mycorrhiza essentially increased only the P uptake, which in turn resulted in a more vigorous plant with a more extensive root system and thus a greater nutrient uptake.

Microscopic observation of the stained root samples showed that the inoculated plants were highly infected with mycorrhiza at the intermediate P levels, but with a low degree of infection at both the very high and very low rates of P application (**Table 2**). In the sterilized soil the non-inoculated plants were essentially free of any mycorrhizal infection, as expected. However, in the unsterilized soil no infection could be observed either, which is surprising in view of the comparatively good growth and P uptake at intermediate P rates in this treatment. This might be due to the presence of some indigenous strains of mycorrhiza with extremely fine hyphae and essentially no vesicles in the roots, as has been found recently in other crops.

Table 2. Effect of soil sterilization on percent infection of roots of cassava, cv. MAus10, inoculated with mycorrhiza and grown for two months in an Oxisol at P application rates of 0 to 16 t/ha.

P applied (t/ha)	Unsterilized soil (% infection)	Sterilized soil (% infection)	P applied (t/ha)	Unsterilized soil (% infection)	Sterilized soil (% infection)
0	0	5	2	53	77
0.1	14	49	4	61	45
0.5	38	79	8	9	57
1	51	65	16	4	14

Many researchers (Hayman, 1975; Sanders, 1975; Zaag *et al.*,1979, Yost and Fox, 1979) have reported that the beneficial effect of mycorrhizal associations decreased as the P concentration in the soil increased, and that at extremely low P levels the association is also not effective (Mosse *et al*., 1975). Similar results were obtained in this study. Zaag *et al*. (1979) reported that the beneficial effect of mycorrhiza in cassava reduced to about zero at P concentrations in soil solution above 52 µM, determined with the method of Fox and Kamprath (1970). In this study, inoculation was effective in increasing yields in the range from 2 to 50 µM P in soil solution, which corresponds with the data from Zaag *et al*. (1979). It is also clear that mycorrhiza do not significantly change the plant's external P requirement as the mycorrhizal effect essentially disappears at the high P concentrations necessary for near-maximum yields. The external P requirement obtained in this trial for all mycorrhizal treatments was about 100 µM (Howeler, 1980), which is not too different from the P requirement of 72 µM obtained for the same cultivar in nutrient solution by Jintakanon *et al*. (1982).

A similar experiment was conducted in the greenhouse at CIAT-Colombia, using a sterilized and unsterilized soil from CIAT-Quilichao (Howeler *et al*., 1982b). This very acid soil (pH 4.3, Al 2.8 meq/100 g) has 7.1% OM, but is highly P-fixing and has a low available P content (Bray II) of 1.8 ppm. Prior to sterilization the soil was incubated for six weeks with nine levels of P, applied as $Ca(H_2PO_4)_2.H_2O$, as well as the equivalent of 1 t/ha of dolomitic lime and fairly high levels of N, K, Mg and Zn. The P levels ranged from zero to 3,200 kg P/ha. Cassava tip cuttings of cv. MMex 59 were rooted in small peat pots in misting chambers, after which they were transplanted to the pots with soil; at this time half of the plants were inoculated by placing 2 g of VAM-infected cassava roots under each tip cutting. The non-inoculated plants received the same amount of dead inoculum.

After three months of growth, the top growth was dried, weighed and analyzed, while the fibrous roots were stained for observation of the degree of VAM infection.

After two weeks of growth there was a clear response to P applications, and at three weeks to mycorrhizal inoculation. In the sterilized soil the non-inoculated plants remained small with typical symptoms of P deficiency up to the level of 1,600 kg/ha, while maximum plant growth was reached at 3,200 kg/ha of applied P. In the inoculated plants, however, there was no visual response to P and the P-check plants appeared as vigorous as the non-inoculated plants with 3,200 kg P/ha. Mycorrhizal responses were most dramatic at low and intermediate levels of applied P (**Photo 3**).

Figure 4 shows that non-inoculated plants required 3,200 kg P/ha to reach maximum DM yield, and even with 800 kg P/ha plants remained extremely small and P deficient. Inoculated plants showed only a minor P response to 200 kg P/ha, and plants to which no P had been applied produced the same DM yield as non-inoculated plants with 1,600 kg P/ha. In the P-check inoculation increased DM yield from 0.42 to 34.6 g/plant, which is a 80-fold increase. Inoculation not only increased the dry weight but also the P concentration of the tissue, even at the highest level of applied P, as shown in **Table 3.**

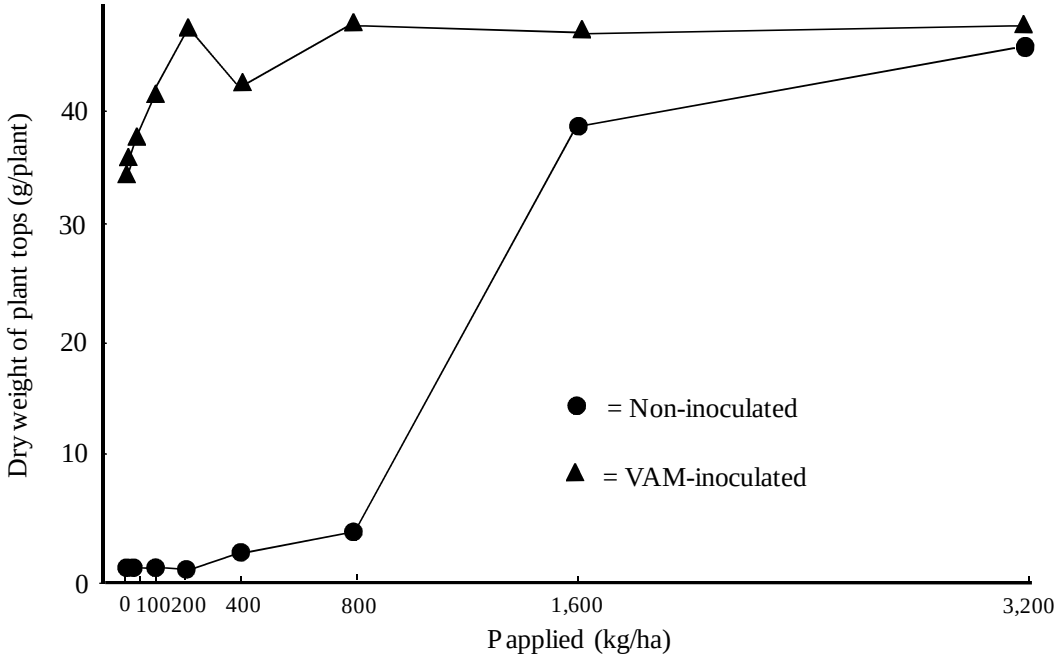

Figure 4. Effect of mycorrhizal inoculation and P application rates on the dry weight of plant tops of cassava, cv. MMex 59, in a sterilized soil from CIAT-Quilichao in the greenhouse. ***Source****: CIAT, 1981.*

Inoculation tended to decrease the K, N and Zn concentrations of the tops, mainly due to better growth and dilution of nutrients in the tissue. The total uptake of nutrients increased markedly due to inoculation, but mainly due to increases in DM. The total P

uptake increased at all levels of applied P, but was marked in the P-check and at low levels of applied P where inoculation increased P uptake over 100-fold. **Table 3** also shows the effect of inoculation and P applications on the degree of mycorrhizal infection. In the inoculated treatments, both the infection and the effectiveness in terms of P uptake were highest at intermediate levels of 50 and 100 kg P/ha. These are levels of P application that often give maximum yields in field experiments (CIAT, 1982) and that are economically feasible for many farmers. The observation of maximum effectiveness at intermediate levels of P application corresponds with similar results obtained in Australia (shown above), as well as that reported by other workers.

Table 3. Effect of P application on P concentration and total P absorption of tops, and on mycorrhizal infection of roots of inoculated and non-inoculated plants of MMex 59 grown in sterilized Quilichao soil.

P application (kg/ha)	P concentration in tops (%)		P absorption in tops (mg/plant)		Root infection rating[1)]	
	Non-inoc.	Inoculated	Non-inoc.	Inoculated	Non-inoc.	Inoculated
0	0.05	0.08	0.2	27.7	0	1.7
25	0.07	0.07	0.5	25.5	0	2.2
50	0.04	0.11	0.3	41.3	0	2.6
100	0.05	0.12	0.3	49.4	0	2.6
200	-	0.17	-	81.0	0	2.4
400	0.06	0.17	1.2	73.0	0	2.0
800	0.09	0.17	3.4	86.0	0	1.5
1600	0.15	0.16	58.3	75.3	0	1.0
3200	0.20	0.25	90.5	118.3	0	1.0

[1)] Visual evaluation of hyphae and vesicles: 0 = no infection; 3 = high infection
***Source**: Howeler et al., 1982b.*

In another similar experiment conducted in the CIAT greenhouse the response to inoculation was determined in both sterilized and unsterilized soil from Carimagua and from CIAT-Quilichao. **Figures 5** and **6** show that in both soils there was a good DM response to P application, reaching maximum yields at 3000 kg P/ha in Carimagua and at 1000 kg P/ha in Quilichao soils. In both sterilized soils there was a marked response to inoculation, increasing shoot weight 15 times in Carimagua and 31 times in Quilichao soil at 100 kg P/ha applied.

In both soils inoculation also markedly increased the P concentration of tops resulting in an increase in total P uptake of 66-fold for Carimagua and 92-fold for Quilichao soils at 100 kg P/ha. However, in the unsterilized soil, there was no significant effect of inoculation in the Quilichao soil, but a marked effect in the Carimagua soil. In the latter inoculation increased dry weight of tops three fold at 100, but only 38% at 300 kg P/ha. The lack of response in unsterilized Quilichao soil is probably due to the fact that plants were inoculated with a strain collected from the same site. Thus, the introduced strain was the same as the dominant native strain, and placement of infected root inoculum did not result in a better root infection than that obtained from spores in the unsterilized soil. In contrast, in the Carimagua soil the introduced strain from Quilichao was more effective than the local strains, resulting in a positive response to inoculation, at least at the three lower levels of applied P. Thus, the effect of inoculation in unsterilized soil is highly

dependent on the effectiveness and the competition from local strains. **Table 4** shows that in the unsterilized soils inoculation did not increase the P concentration of the tops, except at the highest level of applied P. Thus, the total P uptake of inoculated plants was significantly higher in the sterilized soil than in the unsterilized soil. This may be due to a lack of competition of the introduced strain with native micro-organisms, or to the presence of soil pathogens in the unsterilized soil.

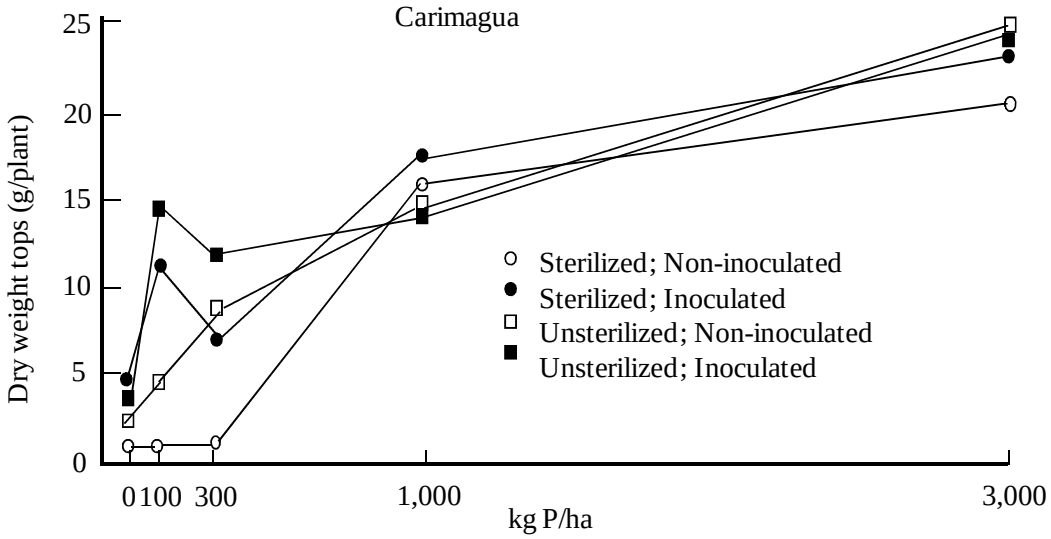

Figure 5. The effect of applied P and mycorrhizal inoculation on the dry weight of cassava grown in pots with sterilized and unsterilized soil from Carimagua.

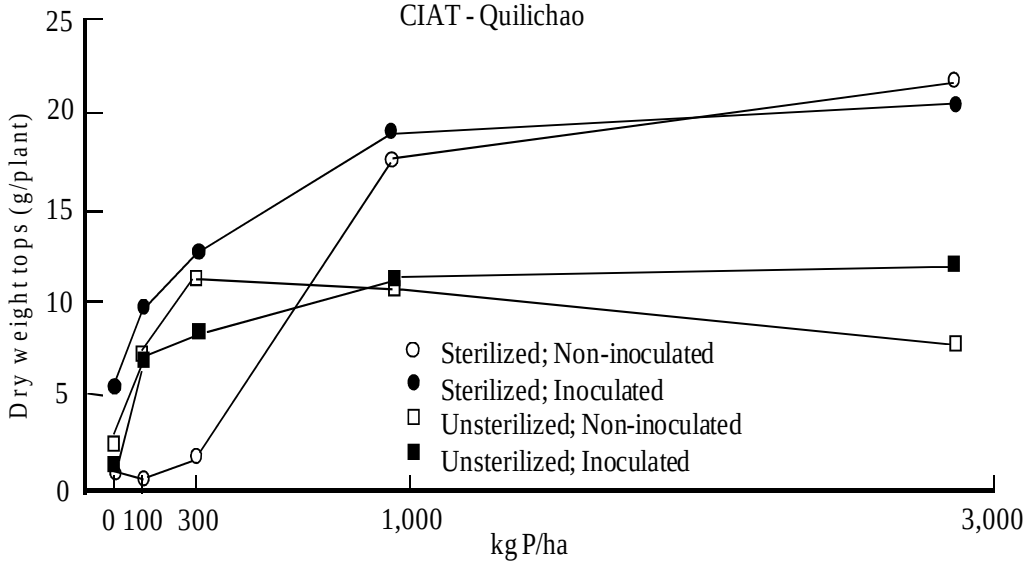

Figure 6. The effect of applied P and mycorrhizal inoculation on the dry weight of cassava grown in pots with sterilized and unsterilized soil from Quilichao.

Table 4. Effect of P application on P concentration and total P absorption of tops of inoculated and non-inoculated plants of CM 91-3 grown in sterilized and unsterilized Carimagua and Quilichao soils.

Soil and P application (kg/ha)	P concentration in tops (%)				P absorption by tops (mg/plant)			
	SN[1)]	SI	UN	UI	SN	SI	UN	UI
Carimagua								
0	0.08	0.14	0.14	0.13	0.54	6.37	3.30	5.08
100	0.07	0.31	0.25	0.17	0.51	33.98	11.07	24.16
300	0.08	-	0.20	0.17	0.75	21.18	16.86	19.75
1000	0.25	0.25	0.17	0.17	39.40	43.05	25.14	24.53
3000	0.34	0.27	0.22	0.27	69.53	62.50	54.45	63.85
Quilichao								
0	0.09	0.17	0.15	0.15	0.27	8.62	1.89	0.90
100	0.09	0.27	0.16	0.16	0.28	25.68	11.20	10.97
300	0.11	0.26	0.23	0.16	1.89	32.40	25.41	13.12
1000	0.16	0.24	0.17	0.18	27.71	44.04	17.51	18.72
3000	0.29	0.29	0.20	0.25	62.90	58.67	15.24	25.45

[1)] SN = sterilized soil, non-inoculated plants; SI = sterilized soil, inoculated plants
UN = unsterilized soil, non-inoculated plants; UI = unsterilized soil, inoculated plants
***Source**: Howeler et al., 1982b.*

It is now well known that cassava obligately depends on VA mycorrhizal fungi for its P nutrition and that an effective mycorrhizal association is required for P uptake and plant growth in nearly all soils. It is also known that the beneficial effect of the mycorrhizal association tends to decrease as the P concentration in soil solution increases. The VA mycorrhizal fungi take up P from the same sources which are also available to the plant roots (Mosse, 1981). Thus, an increase of available soil P due to fertilization would be expected to increase the P uptake ability of the mycorrhizal root system of cassava. However, in many trials it was found that cassava yield responses to increasing P fertilization was highly dependent on the trial site. Over the years many different species of VA mycorrhiza have been found in different soils and on different crops. These species may differ in their adaptation to different soil or climatic conditions.

A greenhouse experiment was conducted at CIAT-Colombia to determine whether the site specificity for P response was related to the composition of the native mycorrhizal population (Sieverding and Howeler, 1985). A sterilized soil from Quilichao was inoculated with pure pot cultures of infected soil containing an equal number of spores of three different VAM species, i.e. *Glomus manihotis*, *Glomus ocultum* and *Entrophospora colombiana*, as well as a mixture of these three species. The soil had previously been incubated with four levels of applied P, i.e. the equivalent of 0, 50, 100 and 200 kg P/ha as triple superphosphate. Rooted tip cuttings of cassava, cv. MVen 77, were planted, one in each pot. After two months of growth the plant tops were harvested and the fibrous roots were stained to determine the total root length as well as the mycorrhizal root length in terms of either the presence of hyphae, arbuscules or vesicles, or of vesicles alone.

Table 5 shows that species of mycorrhizal fungi differed in their effectiveness to enhance growth and P uptake of cassava depending on the P level applied. When the three fungal species were mixed in the inoculum, at the 50 kg P/ha level the P uptake was similar to that of plants inoculated with *E. colombiana* alone; at the highest P level of 200 kg P/ha the P uptake was similar to those plants inoculated with *G. manihotis*. Total root length and infected root length of the plants had an optimum at 50 and 100 kg P/ha depending on the fungal species. The percentage mycorrhizal infection only increased with P applications in plants inoculated with *G. manihotis.* This species, first encountered in Quilichao, is known to be one of the most effective species for increasing cassava growth on acid soils.

Table 5. Effect of increasing P application on shoot dry matter and P uptake of cassava, cv. MVen 77, inoculated with different species of mycorrhizal fungi and grown in sterilized soil from Quilichao in the greenhouse.

Species of mycorrhizal fungi	Dry matter (g/plant) P application rate (kg/ha)				P uptake in tops (mg/plant) P application rate (kg/ha)			
	0	50	100	200	0	50	100	200
G. manihotis	1.55	9.02	10.93	13.73	2.01	13.31	22.61	31.91
G. occultum	2.05	7.49	9.32	11.21	2.77	10.64	13.41	18.23
E. colombiana	2.77	14.44	15.13	14.21	2.87	17.64	21.14	27.91
Mixture[1)]	2.53	15.52	14.20	14.89	2.68	18.09	26.78	30.91
LSD 5%			2.40				3.96	

[1)] Mixture of the three species.
***Source**: Sieverding and Howeler, 1985.*

3. Cassava response to mycorrhizal inoculation in field experiments

Since mycorrhizal inoculation can only be practical in the field if it can have significant effects on the yield of cassava roots from plants grown from stakes in unsterilized soil and harvested after 10-18 months, field experiments were conducted in several locations in Colombia to see the effect of mycorrhizal inoculation on the growth and yield of field grown cassava, either in sterilized or unsterilized soils (Howeler *et al*., 1982b; Howeler and Sieverding, 1983; Sieverding and Howeler, 1985). These experiments were conducted mainly in Quilichao and Carimagua, using essentially the same methodology, which is described below. Quilichao is located in the southern tip of the Cauca valley at about 1000 masl, while Carimagua is located in the Eastern Plains at about 300 masl. In Carimagua the experiments were located in two different sites, a clay loam in Yopare and a sandy clay loam in Alegria. .The soils in all three locations are extremely acid and very low in P as well as most other nutrients, especially in Carimagua, as shown in **Table 6.**

Table 7 shows the VAM infectivity of these soils, expressed as the number of mycorrhizal propagules/100 g soil, as determined by the "most probable number" method reported by Porter (1979). This table also indicates the dominant native mycorrhizal species at each site, as well as their relative effectiveness for increasing cassava growth in sterilized soil. It is clear that the soil in Quilichao has a large and very effective native

VAM population, while the soils in Carimagua have a much lower VAM population, which is also rather ineffective in Yopare but much more effective in Alegria.

Table 6. Chemical and physical characteristics of three soils in which field experiments were conducted.

Location	pH	Organic matter %	Avail. P (Bray II) ppm	meq/100 g Al	Ca	Mg	K	Texture
CIAT-Quilichao	4.3	7.1	1.8	2.8	1.80	0.70	0.18	clay loam
Carimagua-Yopare	4.3	2.3	1.6	2.4	0.22	0.07	0.07	clay loam
Carimagua-Alegria	4.6	2.4	0.9	1.4	0.09	0.05	0.04	sandy clay loam

Table 7. Infectivity and effectiveness of the native mycorrhizal population of three soils in which experiments were conducted.

Location	Infective mycorrhizal propagules per 100 g	Native mycorrhizal species	Effectiveness[1)] in sterilized Quilichao soil
CIAT-Quilichao	2506	*Entrophospora colombiana*	XXX
		Glomus occultum	XX
		Acaulospora appendicula	XX
		Glomus manihotis	XXX
Carimagua-Yopare	171	*Entrophospora colombiana*	XXX
		Gigaspora albida	X
		Acaulospora appendicula	XX
		Gigaspora pellucida	X
Carimagua-Alegria	72	*Acaulospora longula*	XXX
		Entrophospora colombiana	XXX
		Gigaspora fasciculatum	XX
		Glomus manihotis	XXX
		Gigaspora sp.	Not defined

[1)] Evaluation from several greenhouse tests for growth response of cassava to inoculation: X = growth not different from non-inoculated controls; XX = different from controls, not different from overall trial mean; XXX = better growth than trial mean.

Table 8 shows the fertilization and cultural practices used in the trials. Unless otherwise indicated, P was applied as Huila rock phosphate, broadcast and incorporated before planting. Cassava stem cuttings were planted vertically at a spacing of 80 x 80 cm, and N, K and Zn fertilizers were applied at time of planting in a short band to the side of each stake. Various amounts of inoculum consisting of either roots or soil-root mixtures were placed directly under the cassava stakes at time of planting. Except where specified this inoculum contained spores and hyphae of VAM isolate C-1-1, recently named *Glomus manihotis* (Schenck *et al.*, 1984), which had been isolated from cassava roots in Quilichao. This isolate is highly effective on a range of crops and different soils (CIAT, 1981; 1982). It was multiplied in sterilized soil in the greenhouse on several plant species as indicated

below. All trials had four replications with generally 49 plants per plot. Four experiments were conducted in Quilichao and four in Carimagua, which are briefly described below:

Table 8. Fertilization and cultural practices used in the field experiments.

Experiment	Location	Cultivar	Fertilization (kg/ha)
I	Quilichao	CM 91-3	11 P levels (incorporated as TSP 3 years earlier), 100 N, 50 K, 20 Mg
II	Quilichao	MCol 638 MCol 1684	100 P (incorporated as RP[1)]) , 500 dolomitic lime, 100 N, 75 K
III	Quilichao	MCol 638 MCol 1684 MVen 77 CM 91-3	50 P (incorporated as RP), 1000 lime, 100 N, 100 K, 5 Zn
IV	Quilichao	MCol 638 MVen 77	50 P (incorporated as RP), 1000 lime, 100 N, 100 K, 5 Zn
V	Carimagua-Yopare	MVen 77	0 and 100 P (incorporated as RP), 1000 lime, 150 N, 150 K, 10 Zn
VI	Carimagua-Yopare	MVen 77	100 P (incorporated as RP), 1000 lime, 150 N, 150 K, 10 Zn
VII	Carimagua-Yopare	MVen 77	0, 50, 100 and 200 P as various sources, 1000 lime, 150 N, 150 K, 10 Zn
VIII	Carimagua-Alegria	MVen 77	50 P (incorporated as RP), 500 lime, 100 N, 100 K, 5 Zn

[1)] RP = Huila rock phosphate with 8% P, of medium solubility.

Experiment I. Soil sterilization and inoculation in Quilichao

A trial was planted in CIAT-Quilichao to determine the mycorrhizal effects in field grown cassava. The experiment was established on plots which three years earlier had received 11 levels of P, ranging from 0 to 1,130 kg P/ha as triple superphosphate (TSP) broadcast and incorporated. At that time beans (*Phaseolus vulgaris*) were planted for one semester after which the plots remained in grass fallow. The residual effect of P application on the available P in the soil at time of cassava planting is shown in **Table 9**. Half of each plot was covered with plastic and methyl bromide was injected under the plastic at a rate of 0.1 kg/m^2 to kill all microorganisms, including all native mycorrhiza. Mycorrhizal root inoculum was collected from highly infected cassava plants in a nearby field; these fibrous roots were chopped to 0.5-1.0 cm pieces and 1.5 g of inoculum was placed directly below the stakes of cv. CM 91-3 at time of planting. In addition, plants were inoculated with 100 g sand containing about 35 mycorrhizal spores per g. The same amount of autoclaved sand and root inoculum was used in the non-inoculated treatments. Thus, the experiment had main plots of 11 P levels, subplots of sterilized and unsterilized

soil and subsubplots of inoculated or non-inoculated plants. Cassava stem cuttings were planted vertically at a distance of 80 x 75 cm and each plant was fertilized with the equivalent of 100 kg N/ha as urea, 50 kg K/ha as KCl and 20 kg Mg/ha as $MgSO_4.7H_2O$, band applied after planting. At 3 1/2 months, plant height was determined and upper fully expanded leaf (YFEL) blades were sampled and analyzed. At 11 months plants were harvested and fresh root yields were determined. Fibrous roots of each treatment were stained and examined to determine the degree of VAM infection.

Table 9. Residual effect of P application on available P content of soil before planting and the P concentration of YFEL blades of 3 1/2 month old inoculated or non-inoculated cassava plants grown in sterilized or unsterilized soil in CIAT-Quilichao.

P-application (kg/ha)	Soil P (Bray II) (ppm)	%P in YFEL-blades			
		SN[1)]	SI	UN	UI
0	1.8	0.44	0.62	0.41	0.56
33	2.2	0.42	0.48	0.44	0.58
67	2.9	0.39	0.45	0.42	0.46
141	6.8	0.53	0.56	0.41	0.41
253	17.9	0.56	0.62	0.40	0.46
310	25.8	0.64	0.65	0.65	0.59
458	27.7	0.54	0.56	0.56	0.47
603	42.3	0.63	0.62	0.57	0.66
734	56.3	0.57	0.56	0.53	0.61
869	36.3	0.70	0.67	0.57	0.51
1131	117.3	0.46	0.58	0.55	0.53
Average		**0.53**	**0.58**	**0.50**	**0.53**

[1)] SN = sterilized soil, non-inoculated plants; SI = sterilized soil, inoculated plants
UN = unsterilized soil, non-inoculated plants; UI = unsterilized soil, inoculated plants
***Source**: Howeler et al., 1982b.*

Table 9 shows that the application of 11 P levels three years earlier resulted in a range of available soil-P levels from 1.8 to 117.3 ppm. At 3 1/2 months there was a good visual response to inoculation in the sterilized but not in the unsterilized soil In the latter there was only a very minor plant height response to P, even though the low P plots had soil-P levels well below the critical level of 8-10 ppm (Howeler, 1981). In the sterilized soil inoculation increased the average plant height from 44 to 55 cm, while in the unsterilized soil plant height was 48 cm, irrespective of inoculation. **Table 9** also shows that the P concentration in YFEL blades of non-inoculated plants in both sterilized and unsterilized soil increased with increasing levels of P in the soil, but that this was not the case for the inoculated plants. Surprisingly, all treatments, including the non-inoculated plants in sterilized soil, had high P concentrations in the tissue, above the critical level of 0.4%. However, highest P concentrations were found in inoculated plants grown in sterilized soil, which also showed the best plant growth and highest concentrations of K. Nitrogen and Zn concentrations were high but not related to P applications or mycorrhizal treatments. In the sterilized soil responses to inoculation were quite marked (**Photo 4**) up to 5-6 months, after which the non-inoculated plants started to recuperate, first in the borders where plant roots became infected after penetration in unsterilized walkways.

Recuperation then continued throughout the plot probably due to infection from the unsterilized subsoil. At time of harvest at 11 MAP most visual responses to sterilization and inoculation had disappeared.

Root yields and the degree of root infection at 11 months are shown in **Table 10.** There was no statistically significant effect of P applications, but a highly significant overall effect of both soil sterilization and inoculation.

Table 10. Effect of P application on root yield and degree of root infection of 11-month old cassava, CM 91-3, grown with and without inoculation on sterilized and unsterilized soil in CIAT-Quilichao, Colombia.

P-application	Fresh root yield (t/ha)				Degree of root infection[1)]			
(kg/ha)	SN[2)]	SI	UN	UI	SN	SI	UN	UI
0	31	50	33	35	0.6	1.6	0.5	1.0
33	28	51	44	41	0.9	1.2	1.0	0.9
67	32	50	40	40	1.0	1.3	1.0	0.6
141	30	68	40	46	0.6	1.2	0.8	1.5
253	29	43	42	33	1.6	1.3	0.3	1.3
310	55	51	34	32	1.5	1.6	0.6	0.8
458	46	57	42	31	1.0	1.5	1.0	1.3
603	41	54	40	43	1.8	1.3	1.0	0.6
734	37	59	28	37	1.1	1.6	0.6	1.3
869	38	52	41	37	0.5	1.8	1.3	0.8
1131	47	46	33	35	0.9	1.8	0.3	1.3
Average	**38**	**53**	**38**	**37**	**1.0**	**1.5**	**0.8**	**1.0**

[1)] Visual evaluation of hyphae and vesicles: 0 = no infection and 3 = high infection

[2)] SN = sterilized soil, non-inoculated plants; SI = sterilized soil, inoculated plants
UN = unsterilized soil, non-inoculated plants; UI = unsterilized soil, inoculated plants

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Figure 7 shows that in unsterilized soil, averaged over P applications, there was no effect of inoculation, but in sterilized soil inoculation increased yields from 38 to 53 t/ha, i.e. a 40% increase in root yield due to mycorrhizal inoculation. Although this is highly significant, it is still an under-estimation of the importance of mycorrhiza in cassava, because of the recuperation of non-inoculated plants grown in sterilized soil once plant roots reached the unsterilized borders and subsoil. At harvest these plants were equally infected with mycorrhiza as the inoculated plants in unsterilized soil, and even better infected than non-inoculated plants in unsterilized soil (**Table 10**). The inoculated plants in sterilized soil had a significantly higher infection, both in terms of hyphae and vesicles, than other mycorrhizal treatments, and this resulted in greater plant height, higher P and K levels in leaves and ultimately a higher root yield. A better appreciation of the importance of mycorrhiza might be obtained by considering only one P treatment in one replication (**Photo 4**) in sterilized soil. In this case the non-inoculated plants had only a minor root infection and did not recuperate , resulting in a root yield of only 8.7 t/ha compared with 63 t/ha in neighboring but inoculated plants. This is a clear indication that cassava is highly dependent on a mycorrhizal association for P uptake from low-P soils. When grown in these soils cassava can be called “obligate mycorrhizal”.

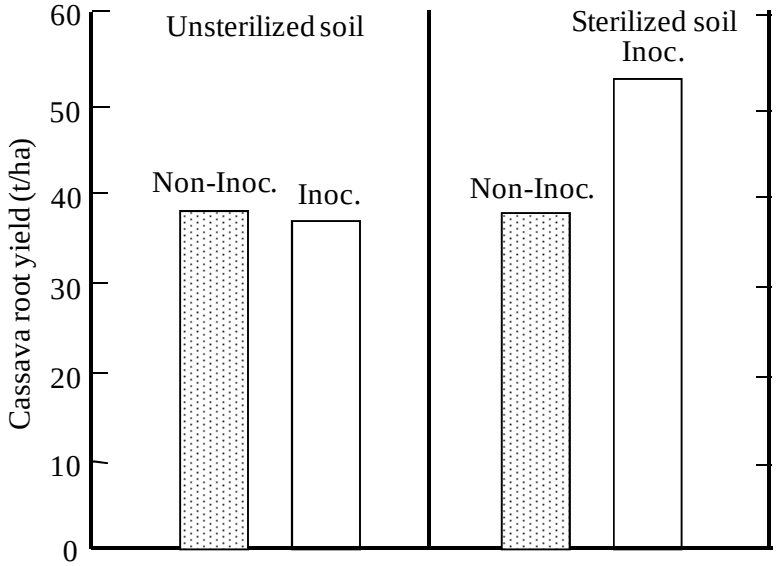

Figure 7. The root yield response to mycorrhizal inoculation of 11-month old cassava, cv. CM 91-3, in sterilized and unsterilized soil in CIAT-Quilichao in 1981. Data are averaged over 11 P treatments.
***Source**: Howeler et al., 1982b.*

The fact that no responses to inoculation were observed in unsterilized soil is mainly because the inoculum used was collected from the same site and therefore must have contained the same VAM species as the native strains present in the unsterilized soil. Among these was a newly identified species, named *Glomus manihotis*, Howeler, Sieverding, Schenck (Schenk *et al*., 1984), which appears to be among the most efficient strains isolated from Colombian soils so far.

Experiment II: Quantity and sources of inoculum in Quilichao

Different levels and sources of inoculum were evaluated in Quilichao in non-sterilized field soil to which 100 kg P/ha as Huila rock phosphate had been incorporated. Only in one treatment the soil was sterilized with methyl bromide two weeks before planting. As inoculum material infected roots of cassava or maize, or soil on which these two crops had been grown, were used. The soil-root mixture of cassava inoculum contained 14 spores of isolate C-1-1 per g, that of maize 29 per g.

Exclusion of the native micro-organisms by soil sterilization inhibited plant growth during the first five months (**Photo 5**). Plants had typical symptoms of P deficiency, indicating the lack of a mycorrhizal association, and some plants actually died of drought stress during the dry season. At three months plant height in sterilized plots was about 30-40 cm, while that in unsterilized neighboring plots was 100-120 cm. After five months, plants in the sterilized plots started to recuperate, first along plot borders and later also in the center. After seven months these plants were actually taller than those in unsterilized plots and showed no further P deficiency. Five months after planting there was still no sign

of root infection in plants grown in sterilized soil, while after 12 months these roots were highly infected. From this it was concluded that native mycorrhizal fungi had invaded the sterilized plots and were highly effective in overcoming P deficiency in the absence of competition from indigenous micro-organisms. However, these plants were physiologically younger at harvest time. **Figure 8** shows the response of cv. MCol 638 and MCol 1684 to soil sterilization and to the average effect of VAM inoculation in unsterilized soil using various sources and amounts of inoculum .

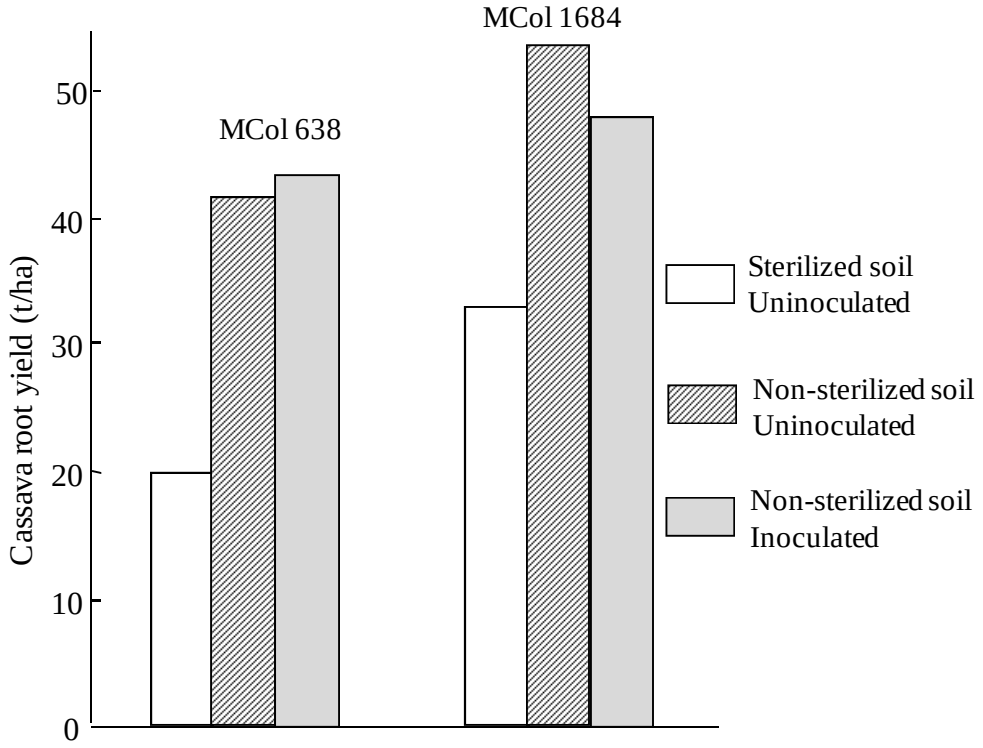

Figure 8. Effect of soil sterilization and mycorrhizal inoculation on the root yields of two cassava varieties grown in Quilichao, Colombia.
***Source**: Howeler and Sieverding, 1983.*

Soil sterilization decreased yields 53 and 39% in cv. MCol 638 and MCol 1684, respectively, compared with non-inoculated plants grown in unsterilized soil. In unsterilized soil there was no significant positive yield response to any level or source of inoculation material, except to that of 2 g infected maize roots in cv. MCol 638. The experiment showed clearly the great dependence of cassava on an effective mycorrhizal association in low-P soils, since the lack of mycorrhizal infection in the early stage of plant development reduced cassava yields to nearly half in comparison to normal infected plants. Similar to the earlier experiment, the lack of a yield response to inoculation in unsterilized soil was due to a very effective indigenous mycorrhizal population, consisting mainly of *Glomus manihotis* and *Entrophospora colombiana*, which so far have been found to be the most efficient species for cassava grown on acid low-P soils.

Experiment III: Inoculation of four cassava cultivars in Quilichao

Four cassava cultivars were planted in CIAT-Quilichao and inoculated with 500 g of a soil-root mixture of maize, which had been produced in the greenhouse. The inoculum contained 2.5 spores of VAM isolate C-1-1 (*Glomus manihotis*) per g.

Inoculation resulted in increased vigor of cvs. MCol 638 and CM 91-3, but at final harvest after 12 months the increase of 11-12% in root yield was not statistically significant (**Table 11**).

Table 11. Root yield (t/ha) response of four cassava cultivars to mycorrhizal inoculation with *Glomus manihotis* (C-1-1) in Quilichao. Numbers in parenthesis indicate the coefficient of variation.

Cultivar	Non-inoculated	Inoculated with C-1-1
MVen 77	32.2 (15.3%)	28.9 (8.0%)
MCol 638	27.1 (24.2%)	30.1 (13.7%)
MCol 1684	41.4 (12.4%)	39.4 (8.0%)
CM 91-3	30.6 (20.9%)	34.4 (8.8%)

In cv. MVen 77 and cv. MCol 1684 there was a slight but non-significant decrease in yield due to inoculation. However, it was noted that inoculation with C-1-1 decreased the mean coefficient of variation from 18.2 to 9.5%, indicating that inoculation resulted in a more uniform root infection, and thus a more uniform growth of the whole plant population. Infection from indigenous endophytes tended to be more erratic probably due to uneven distribution of propagules in the virgin soil.

Experiment IV: Inoculation and mulching in Quilichao

Two cassava cultivars were inoculated as in Experiment II. In half of the experiment the soil was covered with a 10-15 cm layer of grass straw of *Brachiaria decumbens* (15 t/ha).

Application of mulch decreased soil temperature at 10-20 cm depth from 30-32ºC to 25-26ºC (measured at 4 pm) during the first three months of growth. Diurnal temperature fluctuations were also reduced by mulching (25 to 36ºC without and 24 to 26ºC with mulch). Soil moisture was 3.5% higher under mulch. After six months plants grown with mulch were taller than those without mulch, particularly those that had been inoculated.

Figure 9 shows that field inoculation with isolate C-1-1 increased yields more consistently with mulch than without mulch, possibly due to lower soil temperature fluctuations; however, the response to inoculation was not significant. Mulching increased yields significantly in both cultivars and caused a 10 t/ha increase in root yield in cv. MCol 638. As in Experiment II, inoculation resulted in a decrease of the coefficient of variation from 22.4 to 13.6%, indicating that more stable yields may be achieved by inoculation in this soil with a very efficient indigenous VAM fungal population.

Experiment V: Soil sterilization and inoculation in Carimagua-Yopare

Two weeks before planting in Carimagua half of the experimental area was sterilized with methyl bromide at the rate of 1 kg per 10 m^2 in plots that had previously received 0 or 100 kg P per ha in the form of broadcast and incorporated rock phosphate. In

subplots cassava stakes were either not inoculated or inoculated with 100 g of a soil-root mixture of maize infested with isolate C-1-1. Soil sterilization in Carimagua had a similar effect as in Quilichao in Experiments I and II. Plants in sterilized soil without inoculation sprouted but did not grow to more than 30-40 cm, remaining very thin and with typical symptoms of P deficiency.

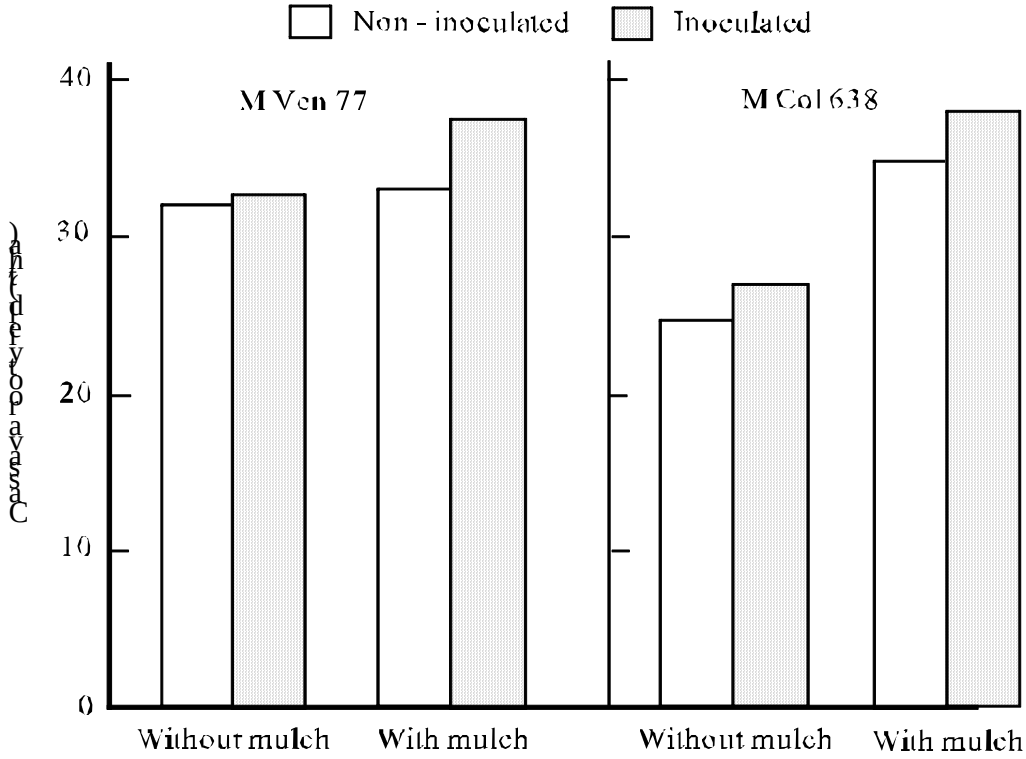

Figure 9. Effect of field inoculation with Glomus manihotis (isolate C-1-1) on the yields of two cassava cultivars, cv. MVen 77 and MCol 638, grown with and without mulching of soil in Quilichao.
***Source**: Howeler and Sieverding, 1983.*

However, inoculated plants grown in sterilized soil were more vigorous than those growing in unsterilized soil. In the latter there was only a minor visual response to inoculation. Again, after seven months uninoculated plants grown in sterilized soil started to recuperate from P deficiency, first along the plot borders and later throughout the plots, but they never attained the vigor and height of plants that were mycorrhizal at an earlier stage.

Figure 10 shows that there was a marked root yield increase due to application of 100 kg P/ha and a negative effect of soil sterilization in non-inoculated plants. As was observed in a similar trial in Quilichao (Experiment I), highest yields were obtained with inoculated plants grown in sterilized soil, either due to elimination of pathogens or competing micro-organisms, or due to the indirect effect of sterilization on micro-nutrient

availability. The latter is not likely since Zn was applied, and other micro-nutrients were never shown to be deficient in these soils. Inoculation in sterilized soil increased root yields nearly 3-fold without applied P and 164% with 100 kg P/ha applied. In unsterilized soil the response to inoculation was not significant without applied P, but significant with 100 kg P/ha. Thus, in this very low-P soil, P application is required to stimulate the effectiveness of isolate C-1-1.

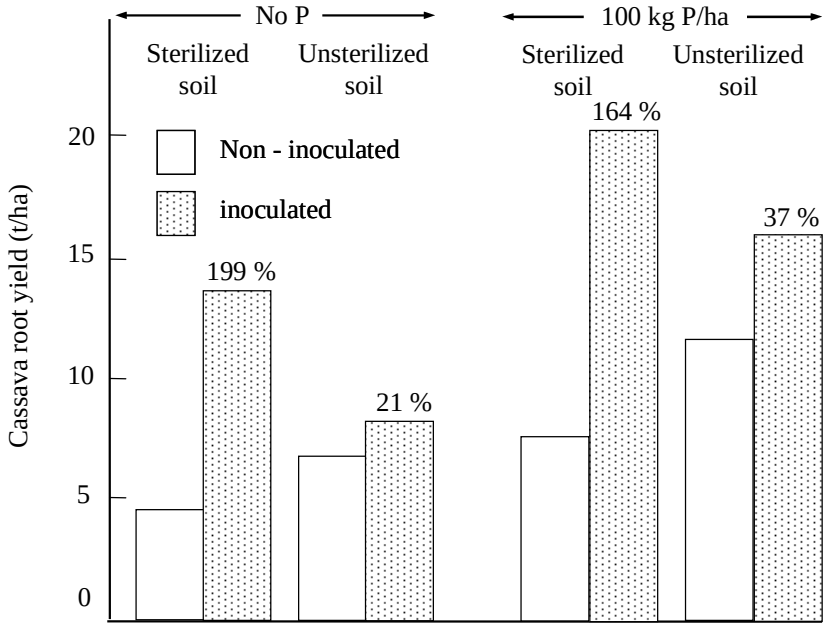

Figure 10. Effect of soil sterilization, P application and mycorrhizal inoculation on the root yield of cassava, cv. MVen 77, grown in Carimagua-Yopare. Numbers on bars indicate the percent response toVAM- inoculation.
Source: *Howeler and Sieverding, 1983.*

Experiment V: Inoculum sources in Carimagua-Yopare

Cassava stakes were inoculated by placing 2 g of chopped-up roots of various plant species under each stake. The inoculum material was collected from potted plants infected with isolate C-1-1 grown in the greenhouse, or from experimental fields in Carimagua which were infested with native mycorrhizal fungi.

Inoculation with chopped-up infected roots of various plant species did not result in marked increases in the vigor of plants grown in unsterilized soil. However, at time of harvest there was a significant response to inoculation with roots of cassava and *Panicum maximum*, both infected with isolate C-1-1 (**Figure 11**).

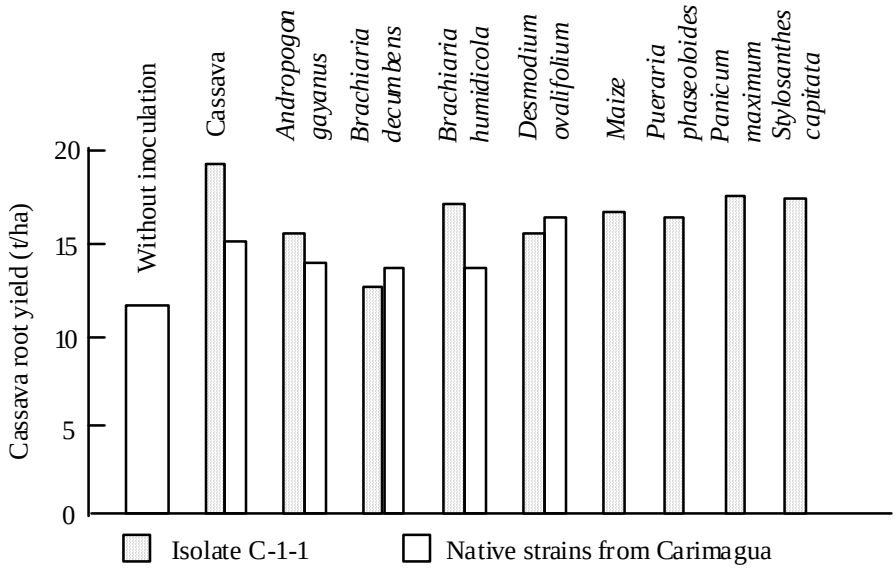

Figure 11. Effect of inoculation with roots of various plant species infected with mycorrhizal isolate C-1-1 (Glomus manihotis) or with native mycorrhiza from Carimagua on the root yield of cassava, cv. MVen77, grown in unsterilized soil in Carimagua-Yopare. ***Source****: Howeler and Sieverding, 1983.*

Inoculation with cassava roots increased yields 64% over the uninoculated control. Inoculation with roots from other species increased yields consistently but not significantly. Thus, it appears that inoculation with this isolate of *Glomus manihotis* was more effective than with local strains, while inoculum of infected cassava roots was more effective than

that of other plant species. Unfortunately, cassava is not a very good species for inoculum production because of the relatively small amounts of roots produced, and the increased possibility of transmitting root diseases when inoculum of the same plant species is used.

Experiment VII: Sources and levels of P application in Carimagua-Yopare

Before planting, five different P sources, i.e. triple superphosphate (TSP), basic slag and rock phosphates (RP) from Morrocco (Reno) and Colombia (Huila and Pesca) were broadcast at levels of 0, 50, 100 and 200 kg P/ha and incorporated. In subplots plants were either inoculated or not-inoculated. The inoculum consisted of 100 g root-soil mixture of maize containing isolate C-1-1.

Incorporation of different levels and sources of P had a rather marked effect on plant growth in a soil which originally had only 1.6 ppm available P. Even so, without P application cassava produced nearly 10 t/ha of fresh roots, while after P application yields increased to a maximum of 17.6 t/ha. **Table 12** shows that root yields increased markedly due to P application but there were no overall significant differences among P levels or sources.

Table 12. Effect of mycorrhizal inoculation and the application of various levels and sources of P on the root yield and starch content of 12 month old cassava, cv. MVen 77, grown in unsterilized soil in Carimagua-Yopare.

	Root yield (t/ha)		Root starch content (%)	
Treatments	Inoculated	Non-inoculated	Inoculated	Non-inoculated
0 P	9.35	9.80	28.7	29.3
50 P - TSP	15.55	13.72	29.2	29.3
- Basic slag	12.45	12.12	30.1	29.4
- Reno RP	13.20	11.55	29.1	29.1
- Huila RP	11.27	9.20	29.3	28.8
- Pesca RP	14.47	12.80	27.7	29.7
100 P - TSP	17.60	11.65	31.0	30.1
- Basic slag	16.62	15.05	30.7	29.3
- Reno RP	16.17	14.22	29.6	29.7
- Huila RP	15.22	13.42	28.8	30.3
- Pesca RP	15.20	10.90	31.2	30.9
200 P -TSP	15.82	15.70	29.9	31.1
- Basic slag	14.62	12.05	30.6	30.9
- Reno RP	14.30	11.22	29.2	30.6
- Huila RP	13.57	12.75	30.6	31.3
- Pesca RP	13.42	15.72	29.5	30.5
Average	**14.30 a**	**12.62 b**		

Inoculation, however, increased yields significantly at a level of 100 kg P/ha, when the average of all P sources are compared (**Figure 12A**). As observed in Experiment V, there was no inoculation response without applied P; the response increased with 50 and 100 kg P/ha and decreased again at the highest level of application. The fact that the

greatest responses were obtained in the field at intermediate levels of applied P corresponds with previous data obtained in greenhouse trials (Howeler *et al.*, 1982a; 1982b), and also with those reported by many other researchers (Daft and Nicolson, 1969; Hayman, 1975; Yost and Fox, 1979).

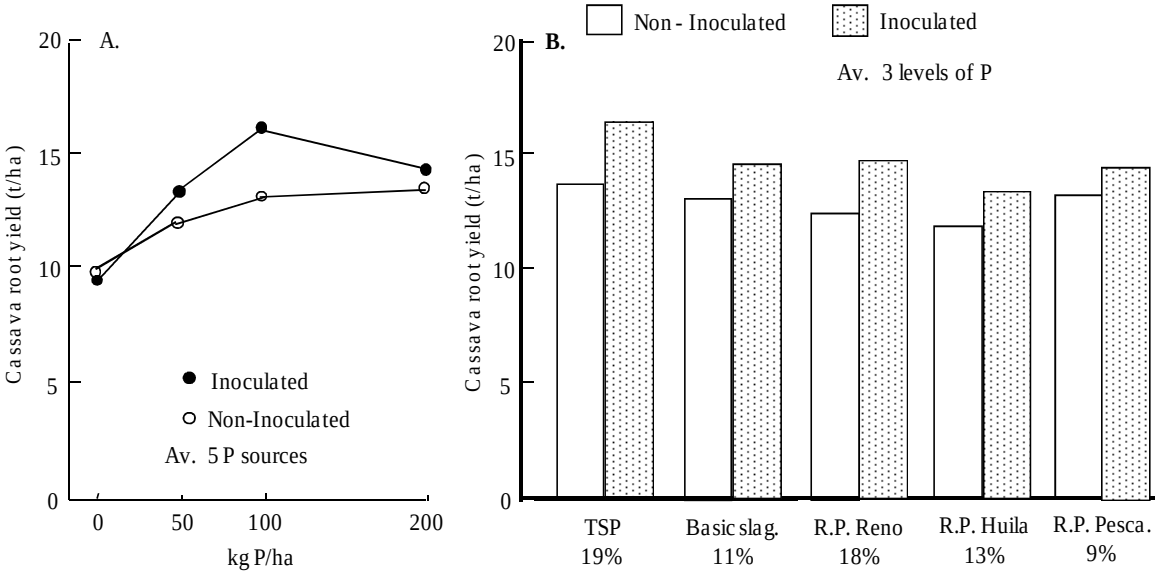

Figure 12. Effect of various levels (A) and sources (B) of P, as well as mycorrhizal inoculation on the root yield of cassava, cv. MVen 77, in Carimagua-Yopare. Numbers below the sources indicate the percent yield increase due to inoculation.

It is clear that mycorrhizal inoculation either increased yield at 100 kg P/ha, or decreased the fertilizer requirement, since the application of 50 kg P/ha with inoculation resulted in the same yield as 100 kg P/ha without inoculation. While there were no significant differences among P sources, the greatest response to inoculation was obtained with TSP, followed by Reno RP. Thus, the common belief (Daft and Nicolson, 1969; Murdoch *et al*., 1967) that mycorrhizal inoculation will be especially beneficial in those soils to which rock phosphates have been applied is not necessarily correct for cassava grown on acid soils, as responses were independent of the solubility of the P sources. At the level of 100 kg P/ha applied as TSP, inoculation increased yield 51%, while the overall increase due to inoculation at this P level was 24%. It may be noted that field inoculation had no effect on the starch content, and thus the quality of the roots (**Table 12**).

Experiment VIII: Evaluation of mycorrhizal isolates in Carimagua-Alegria

In the sandy soil of Carimagua-Alegria the effectiveness of 30 mycorrhizal isolates were evaluated. These had been collected in various locations in Colombia (see **Table 13**) and were multiplied on tropical kudzu (*Pueraria phaseloides* Benth) grown in sterilized Carimagua soil in the greenhouse. Cassava plants were either not inoculated or inoculated with about 400 g infested soil-root mixture. Between each row of five inoculated plants were border rows without inoculation.

Table 13. Effect of field inoculation with mycorrhiza fungal isolates of various origins on cassava yields in Carimagua-Alegria.

Isolate No.	Origin of isolate	Dominant mycorrhizal species in inoculum	Cassava fresh yield (t/ha) Root	Shoot
Control	Not inoculated	native mycorrhiza[1)]	9.1	4.1
1	Quilichao, Cauca	*Glomus manihotis**	10.5	6.1
2	Greenhouse trial	*G. manihotis**	10.9	6.4a
3	Popayan, Cauca	*Entrophospora colombiana**	11.3	5.7
4	Mondomito, Cauca	*Glomus* sp.	11.6	5.7
5	Carimagua, Meta	*Glomus* sp.	13.7a	5.8
6	Carimagua, Meta	*G. fasciculatum*	8.5	4.2
7	Carimagua, Meta	*Acaulospora* sp.	10.8	5.6
8	Carimagua, Meta	*G. fasciculatum*	6.3	3.2
9	Carimagua, Meta	*Gigaspora heterogama*	8.9	4.9
10	Carimagua, Meta	*E. colombiana**	12.1	5.7
11	Carimagua, Meta	*E. colombiana**	11.4	6.4a
12	Carimagua, Meta	*A. longula**	15.2a	6.8a
13	Carimagua, Meta	*Acaulospora* sp.	12.0	6.0
14	Carimagua, Meta	*Acaulospora* sp.	12.3	7.8a
15	Carimagua, Meta	*A. mellea*	10.4	5.3
16	Carimagua, Meta	*A. appendicula*	9.2	5.4
17	Media Luna, Magdalena	*G. manihotis**	10.9	5.3
18	Agua Blanca, Cauca	*G. fasciculatum*	12.6	6.5a
19	Agua Blanca, Cauca	*E. colombiana**	9.1	5.7
20	Carimagua, Meta	*G. manihotis**	12.8	6.6a
21	Rothamsted, England	*G. margarita*	8.8	4.9
22	Bitaco, Valle	*Acaulospora* sp.	8.1	4.1
23	Caucasia, Antioquia	*Acaulospora* sp.	12.0	6.1
24	Ruerto Asis, Putumayo	*Acaulospora* sp.	10.3	5.5
25	San Jose del Palmar, Choco	*Glomus* sp.	15.8a	7.3a
26	Puerto Gaitan, Meta	*G. manihotis**	11.1	5.3
27	Puerto Gaitan, Meta	*G. occultum*	9.4	4.9
28	Puerto Lopez, Meta	*Glomus* sp.	10.6	5.9
29	Quilichao, Cauca	*G. fasciculatum*	9.5	4.9
30	Palmira, Valle	*Glomus* sp.	15.8a	7.4a
LSD 5%			4.5	2.1

[1)] native species: *G. manihotis, G. fasciculatum, E. colombiana, A. longula, Gigaspora sp.*
a = significantly different from control ; * = new species (Schenck *et al*., 1984)

Table 13 shows the origin and classification of the 30 isolates used to inoculate MVen 77, as well as the root and top yields obtained after 12 months. Unfortunately, the experiment was planted at the very end of the wet season and many plants were lost due to drought, resulting in a non-uniform stand and high least significant difference (LSD). Of the 30 isolates all but six increased root yields, but only in the case of four isolates was this increase significant. Yields of tops were increased significantly by inoculation with eight isolates. Three of the four isolates giving significant increases in root yield were different but unidentified *Glomus* species, while the fourth one was a newly named species

Acaulospora longula (Schenck *et al.*, 1984) collected in Carimagua. Since two of the four "efficient" species were actually collected in Carimagua, it seems that cassava yields can be increased by either locally increasing the population of indigenous mycorrhizal fungi or by the introduction of new but well-adapted species. Inoculation with *Glomus manihotis*, which was used in nearly all previous experiments, resulted only in a significant increase in top growth but not that of roots. This may be due to the low level of 50 kg P/ha applied in the form of RP in this trial. In Experiment VII no significant yield increases were observed at this P level either. The Alegria soil is actually lower in P and might therefore require higher levels of P application than the soil in Yopare. Apparently, some isolates are quite effective at these low levels of available P in the soil, while others, like *Glomus manihotis*, may require higher P applications. A future evaluation of isolates should probably be carried out at more than one rate of P application.

GENERAL DISCUSSION

The field experiments described above indicate that none of the experiments in Quilichao resulted in significant yield increases due to inoculation in unsterilized soil, but in Carimagua all experiments produced significant yield increases in at least some treatments. While the Quilichao soil is higher in organic matter and bases, it is not very different in terms of P availability and acidity from the Carimagua soil, at least in Yopare. However, large differences exist in the mycorrhizal fungi population as indicated by the mycorrhizal infectivity and effectiveness shown in **Table 7**. Thus, the virgin Quilichao soil had 15 times more infective propagules than the soil in Carimagua-Yopare, and 34 times more than the soil in Carimagua-Alegria. The indigenous mycorrhizal population in Quilichao is strongly dominated by *Entrophospora colombiana* and *Glomus manihotis*, two very efficient species. In Carimagua-Yopare, in contrast, the low VAM population consists besides *E. colombiana* mainly of rather ineffective *Gigaspora* species and *Acaulospora appendicula.* Thus, it is to be expected that responses to inoculation in Quilichao soil would be less than in soils from Carimagua.

Once a site is selected at which mycorrhiza inoculation might be beneficial, a decision has to be made on the quantity and type of inoculum, assuming that an efficient isolate has already been selected. Experiments II and VI showed a good response to application of 2 g of infected maize, cassava or *Panicum maximum* roots, placed directly under the stake. In the greenhouse infected root material of other species like cowpea or *Andropogon gayanus* were found to be almost equally effective (CIAT, 1982), and it was shown that this type of inoculum could be stored for at least three weeks in a cold room without losing viability. In Experiments V, VII and VIII significant responses were obtained by inoculation with 100-400 g of soils with roots of either maize or tropical kudzu. This type of inoculum is easily produced in pots or beds with sterilized soil-sand mixtures and its preparation is not very time consuming. In a crop like cassava with only 10,000-15,000 plants/ha each plant can be individually inoculated at the time of planting, and no more than 1-1.5 t/ha of inoculum (100 g/plant) would be required. This is similar to the fertilizer requirements in many infertile soils. This quantity can be produced cheaply, although transport over long distances might be expensive. In the latter case inoculation material consisting of infected roots only would be preferable.

It is now recognized that an efficient mycorrhizal association is absolutely essential for good growth of cassava. The native VAM population is often quite effective in

establishing this association, and in this case inoculation has no beneficial effect. Through proper soil management, and selection of agrochemicals that do not destroy the native VAM fungi (CIAT, 1982), the beneficial effects of this natural association can be maximized. It has been observed that when cassava is grown without or with only low rates of P application for several consecutive years in soil with low P availability, root yields tend to increase over time due to a build up of the native VAM population, stimulated by the presence of cassava roots. If a particular soil has a low mycorrhizal population and the local strains are not very effective, then there is a potential for inoculation with more effective strains. Inoculation does not necessarily eliminate the need for P fertilization in acid infertile soils, but it increases the efficiency of P fertilizer utilization. In some soils high yields can be obtained by the combination of inoculation and P fertilization (**Figure 12**). While mycorrhizal inoculation will not be beneficial for all crops or on all soils, there appears to be a great potential for certain crops like cassava, which are highly dependent on a mycorrhizal association, and which are often grown on extremely infertile, or highly eroded soils, with low or inefficient VAM fungal populations.

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CHAPTER 20

SOIL EROSION CONTROL [1]

Reinhardt Howeler[2]

INTRODUCTION

With populations increasing at 2-3% per year in most developing countries, there is an ever more pressing need to increase food production. In the past, the increase in food production was mostly achieved through increases in area cultivated. However, since the best arable land is already under cultivation, the further expansion of agricultural land will be more expensive and the areas brought under cultivation will be ever more marginal in terms of climate, soil fertility and slope. Most of the extension of the agricultural frontier occurs by felling and burning trees in natural forests or by cutting and burning brush and grasses in degraded forests or natural savannas. In forested areas, the ash produced from burning the biomass normally adds sufficient nutrients to the soil to allow 2-3 cycles of food crops to be grown before the land is abandoned again and returned to fallow to restore its fertility. This system of "slash and burn" or "shifting cultivation" agriculture is still practiced mainly in Sub-Saharan Africa, but is also common in parts of South America and Asia. In tropical Asia the system is most prevalent in the outer islands of Indonesia, in Vietnam and India. In Indonesia forests were disappearing at a rate of 400,000 ha per year and in Thailand the forested area was decreasing at a rate of 1.6% per year, according to 1980 data.

Erosion and Land Degradation

The relentless process of deforestation is driven by the high demand for tropical timber and the hunger for new land of landless peasants. Once the logging companies have built roads and extracted the most valuable wood, they are soon followed by landless farmers who fell and burn the remaining trees for the planting of food crops. Due to rapid decline in soil fertility as a result of the soil's exposure to direct sun and heavy rainfall, they must abandon their plots after a few years and open up new areas. The abandoned plots either revert back to secondary forest or brush or grasslands (often cogon grass or *Imperata cylindrica*). As population pressure increases, the fallow period is shortened and the cultivation period enlarged. The removal of the forest cover and undergrowth, which protect the soil from the direct impact of raindrops, will greatly increase the amount of runoff and soil erosion, which in turn leads to soil degradation and reduced water infiltration and storage. This will increase peak water flows in creeks and rivers during the wet season, which may cause flooding, and reduce stream flow during the dry season. After deforestation, the soil on sloping land not only degrades by erosion, but also by the rapid decomposition of soil organic matter and by extraction and leaching of nutrients. The loss of soil fertility will thus affect the growth of vegetative cover and the resulting sparse vegetation will in turn enhance erosion and further aggravate land degradation in a progressive process.

Soil degradation is particularly severe in south and southeast Asia because of extreme population pressure on land, high intensity rains and relatively steep slopes.

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[1] For color photos see pages 771-773.

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Estimates of erosion rates from suspended sediments in rivers indicate that soil losses due to water erosion is much more serious in Asia than in South America or Africa (**Table 1**). Milliman and Meade (1983) calculated that the annual discharge of sediments from the major river systems in continental SE Asia amounts to about 3.2 billion tons, while that of insular SE Asia is almost equally high at 3.0 billion tons. In fact, the rivers of tropical Asia discharge about four times more sediments than those of tropical America, and more than ten times as much as those of Africa. Some of this erosion is due to natural processes, especially in the rather unstable and geologically young Himalayan mountain ranges, but much of it is directly due to, or accelerated by, human activity through deforestation, the intense cultivation of hillsides and the opening of roads in unstable mountain areas.

Table 1. Rates of erosion of the continents.

Continents	Area (10^6 km^2)	Mechanical denudation rate (tons/km^2/year)
Africa	29.81	7.0
Asia	44.89	166.0
Australia	7.96	32.1
Europe	9.67	43.0
North and Central America	20.44	73.0
South America	17.98	93.0

Source: *Modified from data in Strakhov, 1967, cited by Chorley, 1969.*

Land Use in Asia and its Effect on Erosion

The upland ecosystem in Asia has a greater diversity of climates, soils, slopes, natural vegetation and even people than the adjacent lowlands. This also means that there is a greater diversity of land use and cropping systems. Thus, at higher elevations, farthest removed from roads and markets, people are mostly subsistence farmers dedicated to small-scale food production and the collecting of forest products. In contrast, in certain areas closer to markets and with relatively good infrastructure they may be entirely commercial farmers using a high input-output system for producing high value crops like cold climate vegetables, flowers and fruits. Typical examples are the Cameroun Highlands of Malaysia, the Da Lat area of South Vietnam and the Batu area of Malang in Indonesia. These farmers may have the resources as well as the incentives to preserve their valuable soil resources by implementing soil conservation measures such as terracing or bunding. Still, due to the extremely intensive cultivation of the land, erosion can be very severe.

At intermediate elevations with extensive areas of degraded forests and grasslands, farmers may use shifting cultivation to clear and burn new plots for temporary food production, while grazing cattle on communal or government land. The regular burning of these grasslands, in many areas mainly cogon grass, in order to stimulate the sprouting of new shoots for grazing, may cause the most severe erosion.

At lower elevations, most farming is sedentary with little opportunity to open up new land. Average farm size is small, ranging from about 0.3-0.5 ha in Java island of Indonesia to 4-5 ha in Thailand. Most farmers are dedicated to the production of upland food crops such as maize, cassava, mungbean, peanut, soybean and sweet potato. Complicated intercropping systems of cassava + maize + rice, with mungbean, groundnut, soybean or cowpea following the intercropped rice, are very common in Indonesia, while in Thailand and Malaysia the crops are grown mainly in monoculture, each in separate regions

according to rainfall and soil fertility. In the Philippines and southern India, cassava is often grown in either recently established or in older coconut plantations. In Vietnam about 34-40% of cassava farmers grow cassava in intercropping systems, mainly with maize (Pham Van Bien *et al*., 1996). The regular and often intensive land preparation employed for growing these annual food crops can lead to soil losses due to erosion of as much as 500 t/ha/year (Hardjono, 1987). In most cases, soil losses range from 10-100 t/ha/year.

Erosion Processes and Effects on Yields

When rain drops fall at high speed on unprotected soil, they tend to break the soil aggregates into smaller units and disperse the individual clay or sand particles. Soils differ in their susceptibility to erosion (erodibility factor) in having various degrees of resistance to breakdown, or aggregate stability, depending mainly on the texture and soil organic matter (OM) content. Thus, soils of intermediate texture, having a large proportion of silt and fine sand particles, have little aggregate stability, and are most susceptible to erosion. Similarly, soils with little OM and/or low biological activity, or those with a low content of free oxides of Fe and Al are most erodible. Once the aggregates are broken down, the smaller particles may be carried away by running water, causing interrill (or sheet) erosion.

Once the runoff water collects and concentrates into small rivulets, the force of the running water can detach particles, and this may result in rill erosion, which may progress into the formation of gullies. The objective of most soil conservation techniques are 1) to protect the soil from direct rainfall impact by the establishment of either a live or dead (crop residue or mulch) vegetative cover, which can absorb the energy of the impact of raindrops, and 2) to reduce the quantity and slow the speed of the runoff water by improving water infiltration into the soil and to reduce the length or steepness of the slope by contour cultivation, contour ridging, contour grass barriers or hedgerows, and by terracing or bunding.

The erosion process selectively removes mainly the organic matter and certain clay fractions, which provide the soil with its water and nutrient holding capacity. Thus, surface runoff results in a direct loss of potentially soil-stored water as well as that of washed-out nutrients, especially from fertilizers, while soil loss due to erosion removes mainly the most productive part of the soil containing a considerable amount of nutrients, especially organic N, P and S, as well as very important micro-organisms, such as N-fixing bacteria and VA-mycorrhiza. The loss of clay and OM also results in a lower cation-exchange capacity (CEC) as well as a lower water holding capacity. Finally, the physical removal of part of the topsoil reduces the effective rooting depth to underlying bedrock or subsoil layers. This also reduces the water storage capacity of the soil and further exacerbates rainfall runoff and erosion (**Figure 1**).

Thus, erosion results in deteriorating soil physical and chemical characteristics, which in turn affect the soil's productive capacity, with shallow soils or those having an unfavorable subsoil being most affected, and highly demanding crops like maize and soybean being more susceptible to yield declines than less demanding crops like rice, cassava or cowpea. Yield declines due to erosion tend to be greater in Ultisols, Oxisols and some Alfisols with a high content of clay and Al in the subsoil, than in deep and relatively fertile Andosols. Yields are more affected by the loss of the upper-most layer of soil compared with the subsequent loss of deeper layers. Thus, yields declined 3-7% with the loss of the first 1 mm of top soil, and 10-25% with the loss of the subsequent 7 mm of soil

(Marsh, 1971). In Alfisols of India, with average annual soil losses of 40 t/ha (or 5 mm), yields declined 1.25% per year for the first five years and 0.95% during the subsequent

years (Magrath, 1990). In cassava-based cropping systems in Java, annual soil losses of 76-144 t/ha resulted in estimated productivity losses of 3.8-4.7% per year (Magrath and Arens, 1989). Cassava yields in severely eroded soils in Mondomo, Cauca, Colombia, were about 50% of those in adjacent non-eroded soil (Howeler, 1986) (**Figure 2**), but this depended also on the fertilizers used (Howeler, 1987) and the susceptibility of the variety (Howeler, 1991).

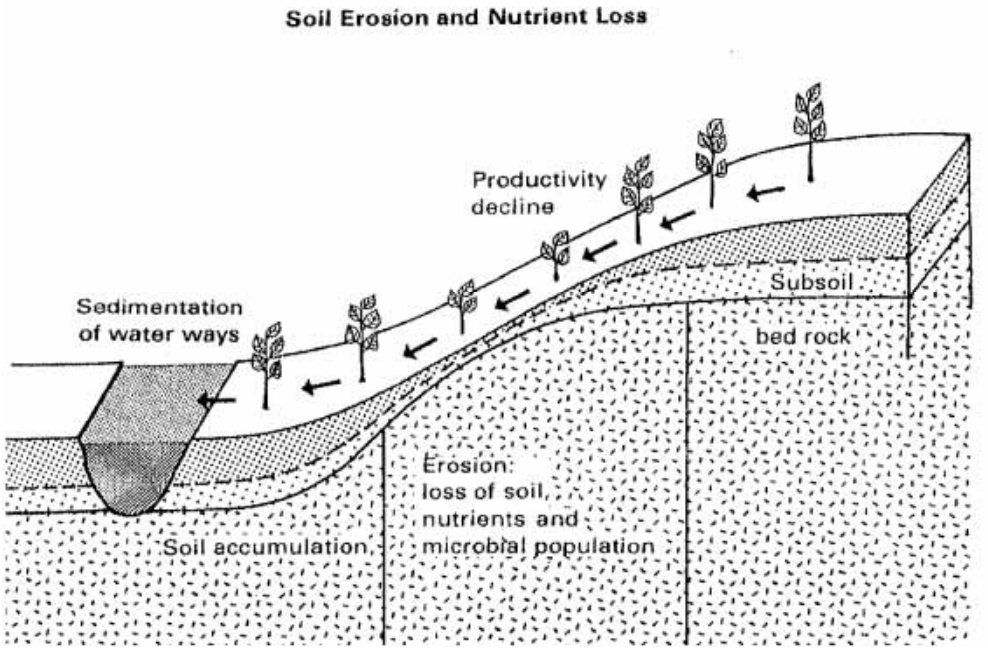

Figure 1. Conceptual representation of the differential effect of erosion in various parts of the landscape on soil depth, nutrient distribution and growth of crops.

On-site and Off-site Costs of Erosion

The greatest cost of erosion is in lost productivity, both present and future. This on-site cost can be in terms of losses of plant stand due to gully erosion and washing out or covering of germinating seed; it can also be in the form of lost productivity due to inadequate soil moisture or nutrients, or due to shallow rooting depth and/or exposure of subsoils. Magrath and Arens (1989) estimated this cost of productivity decline for Java island of Indonesia at 315 million US dollars per year. Besides these on-site costs, soil erosion also has off-site costs in the form of sedimentation of reservoirs and irrigation systems, of flooding of lowlands causing damage to crops and property as well as loss of lives. These off-site costs for Java were estimated to be 26-91 million US dollars per year. Thus, while off-site costs are highly visible and politically sensitive, the on-site costs of erosion, both for the farmer and for the nation, are actually much higher. The main

objective of soil conservation interventions should therefore be to stop erosion on-site in order to prevent losses of soil productivity; this in turn will have a positive side effect in lowland areas.

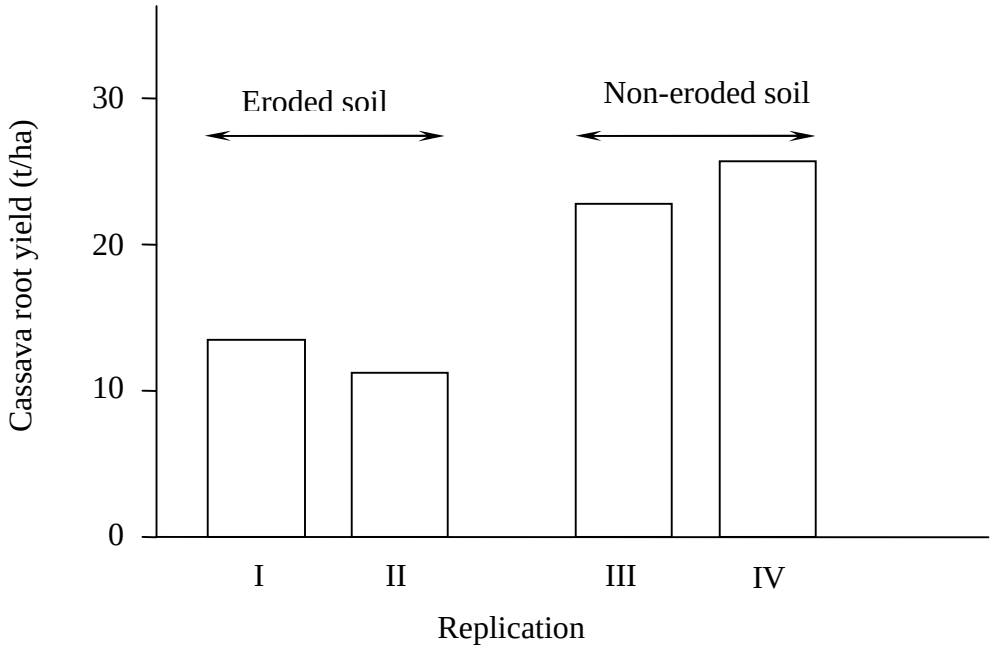

Figure 2. The average yield of 18 cassava varieties planted in two replications on an eroded slope and two replications on an adjacent non-eroded flat area in Mondomo, Cauca, Colombia in 1983/84.
***Source**: Howeler, 1986.*

Factors Determining Soil Loss by Water Erosion

According to the Universal Soil Loss Equation (USLE), soil loss by erosion is a function of the erosivity of the rainfall, the erodability of the soil, the length and gradient of the slope, the crop (C-factor) and management (P-factor). Farmers make the choice of crop and decide about its management; they may also determine to some extent the slope length and gradient by selecting the site of planting within the boundaries of their farm, or they can change the length and slope by contour barriers or terracing. **Table 2** shows average dry soil losses measured in cassava erosion control experiments in seven countries. Even though slope gradients were greatest in Colombia, soil losses were relatively low due to well-aggregated high-OM soils. Erosion losses were highest in Hainan island of China due to high intensity rains in the early part of the growing season when cassava plants grow slowly because of low temperatures in spring. Thus, the extent of erosion is determined by many factors that are beyond the control of farmers.

Table 2. Average dry soil losses due to erosion measured in cassava trials in various countries in Asia as well as in Colombia, S. America.

Country	Site	Slope (%)	Soil texture	OM[1)] (%)	Dry soil loss (t/ha)
China	Xhi Fang, Hainan	8	sandy clay loam	2.4	154
	CATAS, Hainan	15	clay	1.8	128
	CATAS, Hainan	25	clay	2.0	144
	Nanning, Guangxi	12	clay	1.7	16
Indonesia	Malang, E. Java	8	clay	1.5	42
	Tamanbogo, Lampung	5	clay	1.8	47
	Umas Jaya, Lampung	3	clay	2.7	19
Malaysia	MARDI, Serdang	6	clay	-	10
Philippines	Baybay, Leyte	25	clay loam	1.9	54
Thailand	Sri Racha, Chonburi	8	sandy loam	0.6	15
	Sri Racha, farmer's field	8	sandy loam	0.5	18
	PluakDaeng, Rayong	5	sandy loam	0.7	21
Vietnam	Thai Nguyen Univ.	5	sandy clay loam	1.6	23
	Thai Nguyen Univ.	10	sandy clay loam	1.6	39
	Thai Nguyen Univ.	15	sandy clay loam	1.6	105
Colombia	Mondomito, Cauca	27	clay	4.7	45
	Mondomito, Cauca	30	clay	-	2
	Las Pilas, Mondomo, Cauca	40	clay loam	11.0	3
	Agua Blanca, Cauca	42	clay loam	5.1	18
	Popayan, Cauca	15	loam	24.8	15
	Popayan, Cauca	25	loam	24.8	7

[1)] OM = soil organic matter

Source: *Howeler, 1994.*

Crop (C) Factor in the Universal Soil Loss Equation

One way to compare crops or land use systems in terms of their effect on soil erosion is to calculate the C-factor used in the Universal Soil Loss Equation (USLE), as suggested by Wischmeier (1960). In this methodology erosion in a particular crop is measured on (or corrected to) a standard runoff plot of 22 m length and a slope of 9%, and compared with soil losses on a similar but bare plot. The latter is given a value of 1.0, while the C-factor of the crop is a fraction thereof in proportion to the soil losses measured in the crop *versus* that on bare (tilled and weed free) soil.

In order to put the effect of cassava cultivation on erosion in perspective, **Table 3** summarizes C-value data from four sources in the literature. As the data indicate, there is no doubt that natural or planted forests and natural or well-managed grasslands protect the soil better and cause less erosion than annual crops

Table 3. C-values for various land uses and crops calculated by the Universal Soil Loss Equation, as reported by four sources in the literature.

Vegetative Cover/ Crop	C-value			
	1)	2)	3)	4)
Forest				
Primary forest (with dense undergrowth)	0.001	0.001		
Second-growth forest with good undergrowth and high mulch cover	0.003			
Industrial Tree Plantations				
Benguet pine with high mulch cover	0.007			
Mahogany, Narra, eight years or more with good undergrowth	0.01-0.05			
Mixed stand of industrial tree plantation species, eight years or more	0.07			
Agroforesty Tree Species				
Coconuts, with annual crops as intercrop	0.1-0.3			
Leucaena leucocephala, newly cut for leaf meal or charcoal	0.3			
Cashew, mango and jackfruit, less than three years, without intercrop and with ring weeding	0.25			
Oil palm, coffee, cacao with cover crops		0.1-0.3		
Grasslands				
Imperata grassland, well established and undisturbed, with shrub	0.007			
Shrubs with patches or open, disturbed grasslands	0.15			
Savanah or pasture without grazing		0.01		
Grassland, moderately grazed, burned occasionally	0.2-0.4			
Overgrazed grasslands, burned regularly	0.4-0.9			
Guinea grass (*Panicum maximum*)			0.01	
Cover Crops/Green Manures				
Rapidly growing cover crop		0.1		
Velvet bean (*Mucuna sp*)			0.05	
Annual Cash Crops				
Maize, sorghum	0.3-0.6	0.3-0.9	0.05	
Rice	0.1-0.2	0.1-0.2		
Peanut, mungbean, soybean	0.3-0.5	0.4-0.8		
Cotton, tobacco	0.4-0.6	0.5	0.14	
Pineapple	0.2-0.5			
Bananas	0.1-0.3			
Diversified crops	0.2-0.4			
New kaingin areas, diversified crops	0.3			
Old kaingin areas, diversified crops	0.8			
Cassava monoculture		**0.2-0.8**	**0.18**	
Cassava with well-established leguminous ground cover				**0.01-0.02**
Crops with a thick layer of mulch		0.001		
Other				
Built-up rural areas, with home gardens	0.2			
Bare soil	1.0	1.0	1.0	1.0

Sources: *[1] Data from David, 1987, for watersheds in the Philippines.*
[2] Data from Roose, 1977.
[3] Data from Margolis and Campos Filho, 1981, for Pernambuco, Brazil.
[4] Data from Leihner et al., 1996, for Cauca, Colombia.

The perennial plantation crops and fruit trees, like oil palm, cacao, coffee, cashew, mango, jackfruit and bananas have C-values of 0.1-0.3, which is not too different from some annual crops like upland rice or moderately grazed pastures. Other annual crops, like maize, sorghum, peanut, soybean, cotton and tobacco seem to cause slightly more erosion than pineapple, but less erosion than cassava. Cassava has a very wide range of C-values, which indicates that erosion depends mainly on the way the crop is managed, such as plant spacing, fertilizer application or ridging. Leihner *et al*. (1996) actually reported very low C-values, comparable to those of well-managed range land, when forage legumes were grown as a ground cover under cassava. While highly sustainable, this practice is seldom economically viable as the cover crops compete strongly with cassava, resulting in very low cassava yields (see below).

Soil and Water Conservation Practices

Soil and water conservation practices can be separated into two broad groupings, engineering structures and vegetative techniques. In many cases, both are applied at the same time.

Engineering structures

This includes land leveling, the construction of contour banks or bunding and various types of terracing. Although these structural solutions were emphasized in the past, and still play an important role in some countries (especially Indonesia), their cost effectiveness has generally been rather poor. This is due to their high cost of installation ($400-1,000/ha for terraces) as well as high cost of maintenance (Magrath and Doolette, 1990). If terraces or contour banks are not well designed or maintained, they can easily collapse causing severe loss of land. Moreover, drainage ways need to be constructed and maintained to safely conduct the water down slope. Besides the loss of land by terrace risers, there is additional loss of land of 3-5% for drainage ways. Also, depending on slope and soil depths, there may be considerable exposure of infertile subsoils, resulting in reduced productivity or increased fertilizer requirements during the first years after construction. If terraces are built with heavy machinery, this may also lead to soil compaction and extremely high rates of erosion during and shortly after construction. While farmers may construct terraces if given adequate incentives, they will never spontaneously construct terraces because of their high cost, and dubious or only long-term benefits

Vegetative techniques

These include various crop and soil management practices that will provide a vegetative cover of the soil to reduce the impact of raindrops and increase infiltration, or provide barriers to reduce the speed of runoff. Some examples of these techniques are:

- Contour cultivation has been recognized as one of the most effective ways to reduce runoff and erosion, capture soil moisture and increase yields. Compared with the traditional system of up-and-down cultivation, runoff was reduced by 25%, while yields of sorghum increased on average 35% during 30 years of experiments in India (Dhruva Narayana, 1986). On moderate slopes (up to 15%) this can be done by tractor, although it may take more time than up-and-down tillage. On steeper slopes (up to 50%) land can be prepared with oxen- or water buffalo-drawn equipment. A reversible plow, utilized in the Andean zone of Colombia was very effective in contour plowing of steep slopes (Howeler *et al.*, 1993).

- Minimum tillage and/or stubble mulching can be very effective in reducing runoff and erosion. In loose and friable soil, seeds can be planted directly using a pointed stick to make holes, while cassava can be planted by pushing the stakes directly into the soil. In compacted soil or in weedy plots it may be necessary to prepare individual planting spots with a hoe. Another form of minimum tillage is to reduce the intensity of tillage (one plowing instead of various passes with plow or harrow) of the area to be tilled, or alternating contour strips of tilled and untilled soil. While minimum tillage can decrease erosion significantly, it often leads to a reduction in yield due to soil compaction, weed competition and reduced efficiency of fertilizers when these are left on the soil surface. When soils are compacted or the soil surface is sealed by heavy rainstorms, runoff may actually increase and water infiltration decrease.
- Contour ridging was found to be very effective in reducing runoff and erosion on gentle slopes and in stable soil; it often also increases yields by concentrating topsoil in the ridge, increasing rooting depth and conserving soil moisture. However, on steep slopes or with unstable soils, too much water accumulating behind the ridges may cause them to break resulting in concentrated water flow and gully erosion.
- Mulching with crop residues or grass on the soil surface greatly improves water infiltration, protects the soil from direct raindrop impact and reduces runoff and erosion. Mulch application have been shown to increase yields of various crops up to 140% (Suwardjo and Abujamin, 1983) by supplying nutrients, increasing soil moisture during dry spells and reducing soil temperature fluctuations. However, sufficient mulching materials are often not available or their collection and transport is costly. Thus, *in situ* production of mulch by rotating or intercropping food crops with leguminous cover crops may be a more practical solution. Permanent cover crops or "live mulches" of *Calopagonium, Pueraria phaseoloides* or *Macroptillium atropurpureum* have been used successfully for erosion control under perennial trees such as rubber or oilpalm. Attemps to use perennial legumes as cover crops in cassava have been less successful due to severe competition of the cover crops with cassava. Cassava yields were reduced on average 20-50% by nine cover crop species in Thailand (Howeler, 1992; see Chapter 18).
- Vegetative barriers may include:
  1. Contour strips of cut-and-carry grasses such as elephant grass or napier grass (*Pennisetum purpureum*), king grass (*Saccarum sinense*), Bermuda or Bahama grass (*Cynodon dactylon*), Bahia grass (*Paspalum notatum*), etc. These have been used successfully to reduce runoff and erosion and supply feed for cattle or water buffaloes.

     Contour strips of about 1 m width are usually planted at 1-2 m vertical intervals. The drawback of this system is that 15-20% of the land must be taken out of crop production, the grass trimming is labor-intensive , feed production is often more than the family can use, and the grass stolons or feeder roots can seriously reduce yields of adjacent rows of food crops.
  2. Contour hedges of "inert" grasses such as vetiver grass (*Vetiveria zizanioides*) can be very effective in reducing runoff and erosion and may increase crop yields by improved water conservation and reduced nutrient loss. Single row hedges of about 50 cm width are generally sufficient, thus taking less than 10% of land out of production. Moreover, the deep vertical root system of this grass does not compete seriously with adjacent crops. However, the low forage quality of the grass is a serious drawback for those farmers who need to produce animal feed. Also, since the seed of most vetiver grasses are infertile, the hedgerows have to be planted with

vegetative tillers, which are costly to produce, transport and plant, especially in mountainous areas. On the other hand, once planted, the hedgerows can be very effective for many years without the need of replanting.

3. Hedgerows of leguminous trees. The system is generally called "alley cropping" and consists of planting fast-growing leguminous tree species such as *Leucaena leucocephala* or *Gliricidia sepium* in contour lines about 4-5 m apart. Crops are grown in the space between the hedgerows. To prevent light competition the trees need to be pruned regularly to about 30-50 cm height and the prunings can be used as animal feed or placed between the hedgerows as mulch and are a good source of nutrients, mainly N fixed by the trees. While rather labor intensive and slow to establish, this system can eventually be very effective in forming terraces, reducing erosion and increasing yields (see Chapter 18). Basri *et al*. (1990) reported an increase in rice yields of 25-30% by alley cropping with *Cassia spectabilis* in northern Mindanao of the Philippines.

The advantages of these various vegetative techniques are:

- Low cost of installation; barriers of vetiver grass cost only $16 per ha compared with $ 21-80/ha for construction of earthen bunds in India (Magrath, 1990).
- Adaptability: allows for flexible management and does not require much expertise; greater farmer control.
- Less area out of production: about 20-25% for hedgerows in alley cropping systems, but less than 10% for vetiver grass hedgerows.
- No need for water disposal systems, better water retention.
- Natural terrace formation by such practices as contour cultivation, alley cropping and contour grass barriers.
- May provide animal feed by hedgerow trees or grass barriers, or additional income from perennials grown in contour strips; and
- Usable for a wide range of land tenure situations

Many of these vegetative techniques can be applied solely or in combination, and in many cases they act synergistically to increase productivity as well as reduce erosion. However, each technique has its own benefits and its own limitations, which may require certain trade-offs.

To be effective and acceptable to farmers these techniques must:

- Produce direct and tangible benefits to farmers in the form of increased productivity or income
- Require little outside input and have low labor requirements for installation and maintenance
- Be simple and not require expensive machinery or expert advice
- Be adapted to the local conditions for soil and climate, as well as the availability of necessary inputs or markets for outputs; and
- Be effective in soil and water conservation.

In many cases a different choice of crops, a simple change in cropping pattern or time of planting, an increase in plant population or fertilizer application may lead to improved plant vigor resulting in better soil cover, higher yields, improved soil fertility and effective erosion control. Thus, appropriate agronomic practices that increase yields are often the most effective in reducing erosion. Moreover, when intensification of cropping

increases yields and maintains soil fertility, annual crop production can be limited to the permanent cultivation of only the flattest and most fertile part of the farm, leaving the steeper slopes for production of perennial trees, for grazing or forestry. Proper land use planning, diversification and intensification of the farming enterprise will often be the most effective way to control erosion, maintain soil fertility and sustain productivity.

Cassava Cultivation and its Effect on Erosion

Cassava is often considered a crop that causes severe erosion when grown on hillsides. While it is true that the opening of hillsides for cultivation of annual crops will usually increase erosion by several orders of magnitude compared with undisturbed forest or grassland, whether or not cassava causes more erosion than other food crops depends mainly on the circumstances.

Figure 2 shows a summary by Quintiliano *et al*. (1961) of the results of 48 erosion control trials conducted in four experiment stations in Sao Paulo state of Brazil from 1943 to 1959, comparing the effect of different crops and management practices on soil loss by erosion and on runoff.

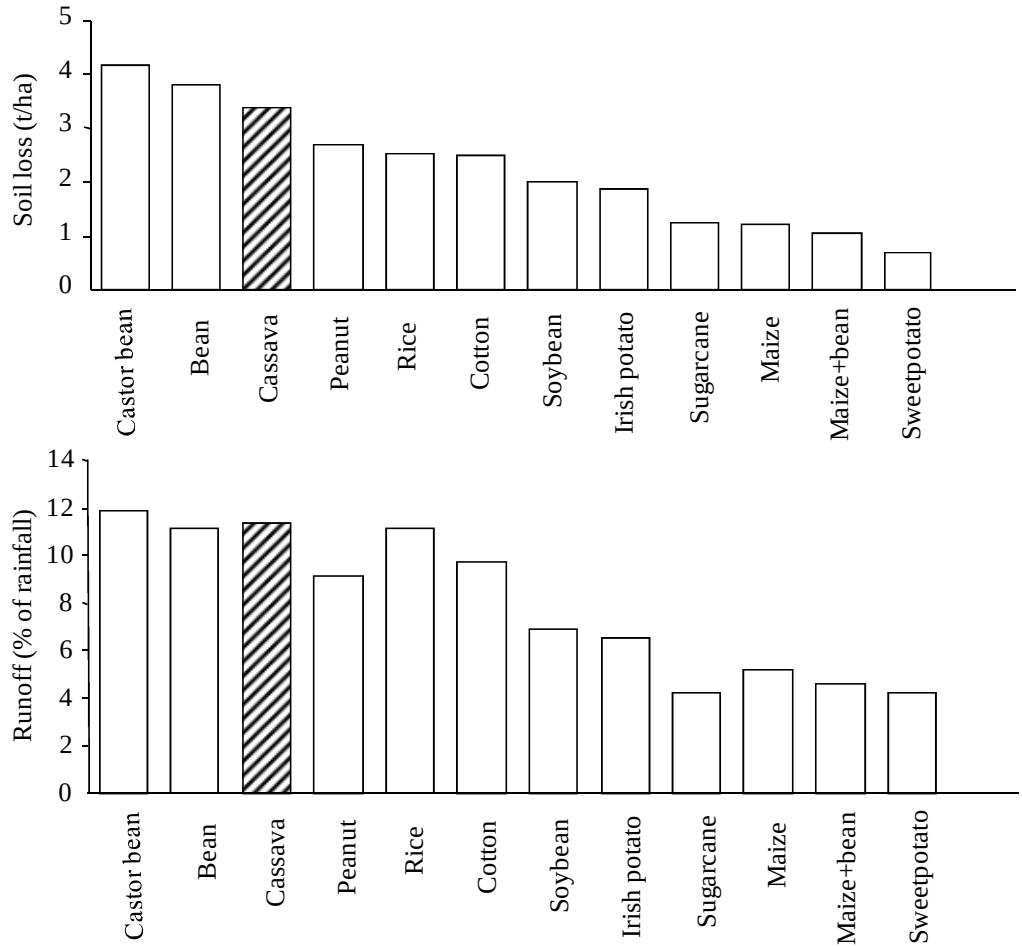

Figure 3. Effect of various crops on annual soil loss by erosion (top) and on runoff (bottom). Data are average values (corrected for a standard annual rainfall of 1.300 mm) from about 48 experiments conducted from 1943 to 1959 on sandy, clayey and Terra Roxa soils in Sao Paulo, Brazil with slopes of 8.5-12.8%. ***Source****: Quintiliano et al., 1961*

Highest soil losses and runoff were observed in castor bean, common bean (*Phaseolus vulgaris*) and cassava, followed by peanut, rice, cotton, soybean, potato, sugarcane, maize and sweet potato. Using the relative soil loss as the criterion, with castor bean considered 100, then cassava would have an index of 83, below that of beans (92), but higher than peanut (64), rice (60), cotton (60), soybean (48), sugarcane (30), maize (29) and sweet potato (16).

In other trials conducted for ten years on 12% slope on a red-yellow Podzolic soil in Pernambuco, Brazil, Margolis and Campos Filho (1981) reported that cassava on average produced an annual soil loss of 11.0 t/ha, compared with 8.3 t/ha for cotton, 3.0 for maize, 2.8 for velvet bean (*Mucuna* sp.) and 0.4 t/ha for guinea grass (*Panicum maximum*), while the soil loss on bare soil was 59.9 t/ha. Although annual soil losses were much higher than those reported by Quintiliano *et al*. (1961), crops are listed in a similar order.

Table 4 shows similar data for soil losses in eight crops planted during four years on 7% slope in Sri Racha, Thailand (Putthacharoen *et al*., 1998). By far, highest levels of erosion were observed in cassava for root production (planted at 1.0 x 1.0 m), followed by cassava for forage production (planted at 0.5 x 0.5 m), mungbean, sorghum, peanut, maize and pineapple. Annual erosion losses for cassava averaged about 75 t/ha, while the average yield was 16 t/ha of fresh roots. Thus, nearly 5 tons of soil were lost for every ton of roots produced. These are extremely high rates of erosion on a slope of only 7%.

Table 4. Total dry soil loss by erosion (t/ha) due to the cultivation of eight crops during four years on 7% slope with sandy loam soil in Sri Racha, Thailand from 1989 to 1993.

Crops	No. of crop cycles	First period (22 months)	Second period (28 months)	Total (50 months)	Average t/ha/year
Cassava for root production	4	142.8 a	168.5 a	311.3	74.7
Cassava for forage production	2	68.8 b	138.5 ab	207.3	49.8
Maize	5	28.5 d	35.5 cd	64.0	15.4
Sorghum	5	42.9 c	46.1 cd	89.0	21.4
Peanut	5	37.6 cd	36.2 cd	73.8	17.7
Mungbean	6	70.9 b	55.3 cd	126.2	30.3
Pineapple[1)]	2	31.4 cd	21.3 d	52.7	12.6
Sugarcane[1)]	2	-	94.0 bc	-	-
F-test		**	**		
cv (%)		11.4	42.7		

[1)] second cycle is ratoon crop; sugarcane only during second 28-month period
Source: *Putthacharoen et al., 1998.*

Erosion losses for cassava in the Thai study were much higher than those of other crops mainly because cassava was planted at a rather wide spacing while initial plant growth was slow, leaving much soil exposed to the direct impact of rainfall during 3-4 months after planting and before the canopy closed. In contrast, the other annual food crops were planted at much higher population densities (50,000-100,000 plants/ha) and had a faster initial growth. Moreover, these row crops were planted along contour lines, which helped considerably in reducing runoff and erosion. Except for mungbean, which was planted six times in four years, all other food crops could be planted only once a year due to

the relatively short (6 month) rainy season in Thailand. Once harvested, the fields remained in weeds with crop residues protecting the soil from further erosion (Putthacharoen *et al*., 1998).

In regions with a longer wet season it is often possible to plant short-cycle food crops, such as maize, rice, soybean, mungbean and peanut, twice a year. In that case, because of more frequent land preparation and weeding, soil losses tend to increase. Comparing one crop of cassava with two successive crops of maize, soybean, peanut and a rice-soybean rotation, Wargiono *et al*. (1998) reported that annual soil losses for cassava were similar to those obtained with two successive crops of soybean, slightly higher than the rice-soybean rotation or two crops of maize, and about twice as high as that of two crops of peanut.

Sheng (1982) reported that in Taiwan, with 2500 mm annual rainfall and on slopes of 20-52%, erosion in cassava was 128 t/ha, compared with 62 for pineapple, 92 for banana, 172 for sweetpotato and 208 t/ha for sorghum, peanut, sweetpotato, soybean and maize grown in rotation. In that case, cassava cultivation resulted in less erosion than the growing of several short-cycle crops in rotation during the same year.

Finally, when four successive crops of beans (*Phaseolus vulgaris*) were grown on 15% and 30% slope in Popayan, Cauca, Colombia, during the same 17 month period as one crop of cassava[1], soil losses for beans in both trials were about four times higher than for cassava, due to the frequent land preparation and weeding required for the beans (Howeler, 1987; 1991). Once the cassava canopy was well established, runoff and erosion losses were greatly diminished; this was also reported by Tongglum *et al*. (1992), Howeler (1995), Tian Yinong *et al*. (1995) and Wargiono *et al*. (1995, 1998).

While slow initial growth and the need for wide plant spacing are intrinsic characteristics of the crop, they can be mitigated against somewhat by planting at a closer spacing, by selecting more vigorous varieties, and by enhancing early growth through fertilizer application. All these have been shown to markedly reduce erosion (see below).

Nutrient Losses in Eroded Sediments and Runoff

Little information exists about the amounts of nutrients lost in eroded sediments and runoff. In most cases where sediments have been analyzed, results are reported as total N (organic + inorganic N), available P and exchangeable K, Ca and Mg. The total loss of P, K, Ca and Mg in the sediment could be an order of magnitude higher than the "available" or "exchangeable" fractions reported. **Table 5** shows results from cassava experiments conducted in Thailand and Colombia. Nutrient losses were a direct function of the amount of soil eroded: practices that reduced erosion automatically reduced nutrient losses. N losses ranged from 4 to 37 kg/ha, while exchangeable K and Mg losses ranged from 0.13 to 5.1 and from 0.1 to 5.4 kg/ha, respectively. Available P losses were considerably lower, ranging from 0.02 to 2.2 kg/ha. As mentioned above, total nutrient losses are considerably higher but no data are available from cassava fields.

[1] Due to the year-round low temperature at about 1800 masl, cassava grew slowly and required 17 months to produce a reasonable yield.

Table 5. Nutrients in sediments eroded from cassava plots with various treatments in Thailand and Colombia.

Location and treatments	Dry soil loss (t/ha/year)	(kg/ha/year) N[1)]	P[2)]	K[2)]	Mg[2)]
Cassava on 7% slope in Sriracha, Thailand[3)]	71.4	37.1	2.18	5.15	5.35
Cassava on 5% slope in Pluak Daeng, Thailand[4)]	53.2	22.3	1.25	3.27	-
Cassava planted on 7-13% slope in Quilichao, Colombia[5)]	5.1	11.5	0.16	0.45	0.45
Cassava with leguminous cover crops in Quilichao[5)]	10.6	24.0	0.24	0.97	0.81
Cassava with grass hedgerows in Quilichao, Colombia[5)]	2.7	5.8	0.06	0.22	0.24
Cassava planted on 12-20% slope in Mondomo, Colombia[5)]	5.2	13.3	1.09	0.45	0.36
Cassava with leguminous cover crops in Mondomo[5)]	2.7	6.5	0.04	0.24	0.20
Cassava with grass hedgerows in Mondomo, Colombia[5)]	1.5	3.5	0.02	0.13	0.10

[1)] Total N

[2)] Available P, and exchangeable K and Mg

[3)] ***Source:*** *Putthacharoen et al., 1998.*

[4)] ***Source:*** *Tongglum et al., 2000.*

[5)] ***Source:*** *Ruppenthal et al., 1997.*

Phommasack *et al*. (1995, 1996) reported total nutrient losses in sediments and runoff from maize fields with 25-35% slope in Luang Prabang, Laos: in the second year of cropping, N, P and K losses in the eroded sediments (9.2 t/ha) were 53.9, 9.3 and 24.0 kg/ha, respectively, while those in the runoff (2,120 m^3/ha) were 2.3, 0.9 and 26.1 kg/ha, respectively (Howeler and Thai Phien, 2000). Although in this case soil loss and runoff were not particularly high, nutrient losses in the sediments and runoff were substantial, especially that of N and K in the sediments and K in the runoff.

Effect of Agronomic Practices on Soil Erosion and Cassava Yields

Soil loss by erosion is mainly determined by the way the crop is managed. The effect of certain cultural practices on erosion is highly site-specific and some practices that are most effective in reducing erosion in one site may not be so at another. This depends mainly on the soil type, the slope, the rainfall pattern, plant type, weeds etc. In many cases there is a conflict, as certain practices may be very effective in reducing erosion, but also cause a reduction in cassava yield. This is generally unacceptable to farmers. It is imperative for farmer acceptance that erosion control practices not be too expensive or labor intensive and not cause a reduction in yield. Ideally they should increase yield.

To determine the effect of various agronomic practices on cassava yields and soil erosion, many erosion control trials were conducted, both in Colombia and in various countries in Asia. Most of these experiments used the simple methodology, described in detail in Chapter 13, in which plots with different treatments are laid out side by side on a uniform slope. Along the lower side of each plot a trench is dug and covered with a sheet of plastic in such a way that the runoff water and sediments eroded from the plot are captured in the trench. The runoff water is allowed to seep away through small holes made in the plastic while the wet sediments remain on the plastic. This wet sediment is periodically removed and weighed and a small sample is dried to determine the dry matter content in order to calculate the dry soil loss per ha. Precautions must be taken that no

water enters the plots from the slope above the trial and no runoff water leaves the plots through the side borders. Some experiments were conducted on experiment stations with replications, but most were conducted on farmer's fields or by farmers with help from researchers or extensionists. The latter normally did not have replications. However, if these farmer participatory research (FPR) trials were conducted with the same treatments by several farmers in the same village, the average results were calculated and presented to show farmers the amount of soil lost and the yields obtained in each treatment. In addition the gross income, total production costs and net income from each treatment were calculated and presented to the farmers, so they could discuss the *pros* and *cons* of each treatment and select and adopt those most suitable for their own conditions.

Land preparation practices

Different methods of land preparation can have a profound effect on both soil erosion and cassava yields as the intensity of land preparation largely determines the aggregate stability of the prepared soil. **Figure 4** shows the results of a land preparation trial conducted on 15% slope in Popayan, Colombia, and planted in separate plots with cassava and *Phaseolus vulgaris* beans. Because of its high elevation (1,800 masl) cassava roots were not harvested until 17 months after planting. During that same period four crops of beans could be planted and harvested. Soil losses in cassava were relatively high during the first six months but leveled off once the crop was well established. In the first bean crop soil losses were minimal, but in the second and subsequent plantings soil losses in the bean plots became extremely severe, especially during the first month after planting, when inadequate plant growth left much of the soil exposed. After 17 months, highest soil losses in bean plots were 105 t/ha, while those in cassava were only 26.5 t/ha. Thus, due to the short growth cycle of beans and the need to prepare the land and replant the crop every four months, soil losses due to erosion were much greater than in cassava, which required land preparation only once every 18 months.

Among the land preparation treatments, soil losses in both crops were highest in plowed plots, while the chisel plow or rototiller caused significantly less erosion. Strip preparation with the rototiller, in which 1 m wide prepared contour strips were alternated with 1 m unprepared strips, was highly effective in reducing erosion in both crops as the unprepared grass-covered strips served as barriers to run-off water. However, **Table 6** indicates that strip preparation caused a significant reduction in both cassava and bean yields, because the actual cropped area was greatly reduced by the unprepared strips, while the grass growing in the unprepared strips may have competed with the crops for water and nutrients.

Another trial on the effect of manual land preparation was conducted at the same time on an adjacent site with 30% slope, again with the same cassava and bean varieties, with very similar results. Again soil losses by erosion were about four times higher with the four crops of beans as with one crop of cassava grown during the same 17 month period. **N**o preparation or hoe preparation of 1 m wide strips alternated with 1 m unprepared strips were most effective in reducing erosion, but these treatments also resulted in the lowest yields. Highest yields of both crops were obtained with complete land preparation with hoe, while lowest yields were obtained with strip preparation. Soil losses due to erosion were much lower in the plots prepared by hoe as compared with those prepared by tractor shown in **Table 6** (CIAT, 1988).

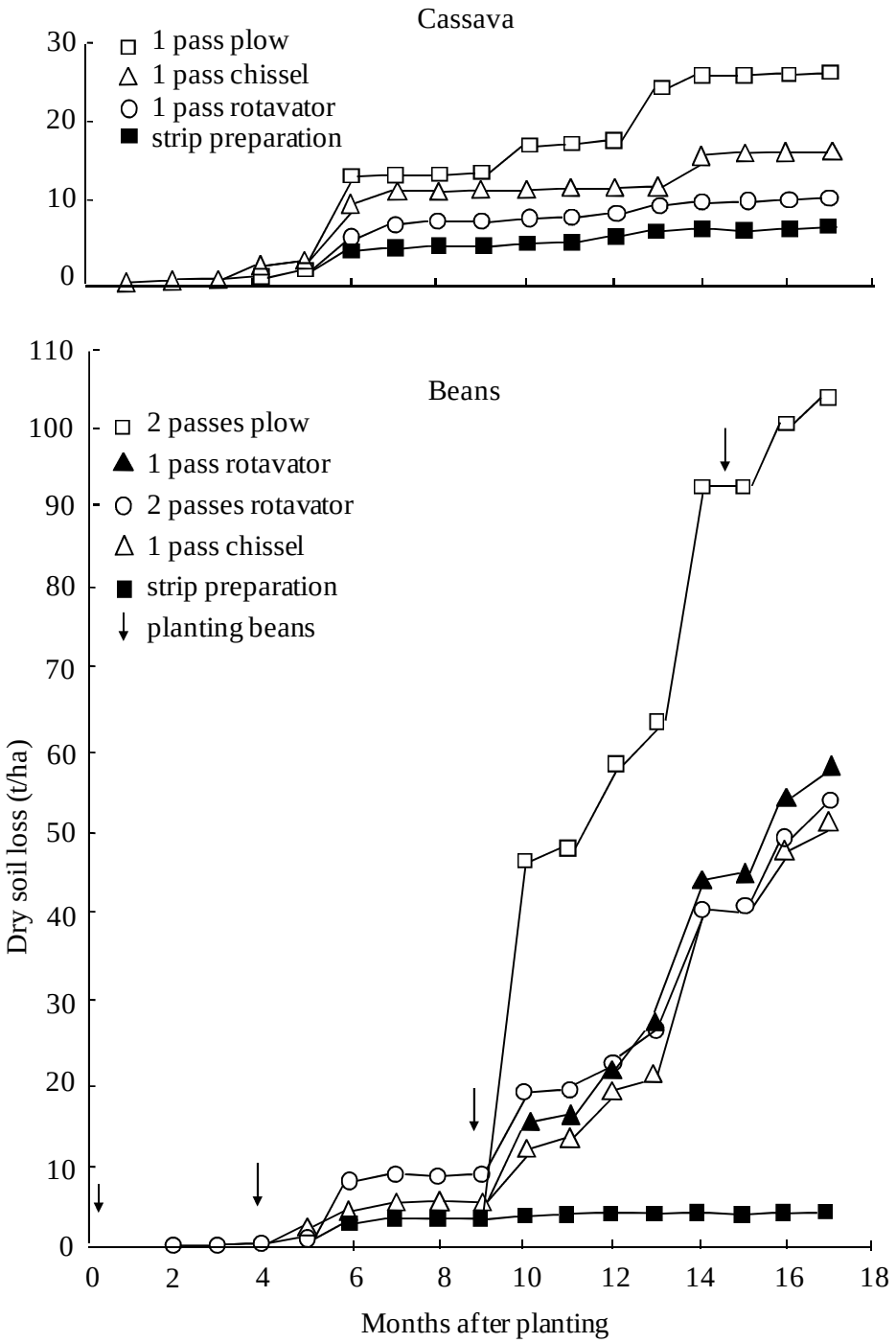

Figure 4. Effect of various mechanical land preparation methods on soil losses due to erosion on 15% slope in Popayan, Colombia, grown with cassava and Phaseolus beans. Arrows indicate when beans were planted.
***Source**: CIAT, 1988.*

Table 6. Effect of mechanical land preparation methods on yields of cassava and beans, as well as on soil loss due to erosion on a 15% slope in Popayan, Cauca, Colombia.

Crop	Method of land preparation	Yield (t/ha) [1)]	Dry soil loss (t/ha) [2)]
Cassava	1 pass with plow	21.4	26.50
	1 pass with rototiller	16.6	10.71
	1 pass with chisels	17.5	16.21
	1 m strips with rototiller alternated with 1 m strips without preparation	12.3	7.50
Beans	2 passes with plow	2.15	104.68
	1 pass with rototiller	2.39	58.78
	2 passes with rototiller	2.89	54.82
	1 pass with chisels	2.20	51.18
	1 m strips with rototiller alternated with 1 m strips without preparation	1.34	4.61

[1)] Cassava fresh root yield after 17 months, average of two varieties; bean yield is sum of three consecutive harvests; fourth crop was lost due to drought and diseases.

[2)] Total dry soil loss in 17 months

Source: *CIAT, 1988.*

Another experiment was conducted on 25% slope at CATAS, Hainan, China, on land preparation methods. **Table 7** shows that complete land preparation, including two times plowing, 2 diskings followed by contour ridging produced the highest yield and an intermediate level of erosion. The same treatment without ridging produced a similar yield but an extremely high level of erosion of 141 t/ha. Planting cassava in hand-made planting holes (30x30 cm) also produced high yields as well as the lowest level of erosion, while planting without any tillage resulted in a low yield and an intermediate level of erosion (Zheng Xueqin *et al*., 1992).

Table 7. Effect of method of land preparation on cassava yields and on dry soil loss due to erosion when cassava was planted on 25% slope at CATAS in Hainan, China in 1989.

	Methods of land preparation	Cassava yield (t/ha)	Dry soil loss (t/ha)
1	Complete preparation: 2 plowing, 2 disking, contour ridging	26.3	71
2	2 plowing, 2 disking, no ridging	26.0	141
3	1 plowing, no ridging	21.3	91
4	4 m wide plowed strip alternated with 1 m strip without prep.	23.5	145
5	2 m wide plowed strip alternated with 0.5 m strip without prep.	22.6	82
6.	Preparation of planting holes with hoe	25.5	45
7	No preparation	22.6	60

In the same trial conducted in the same plots at CATAS in 1991, soil losses were as high as 259 t/ha in treatment 4 due to exceptionally high rainfall in June, July and August, while the lowest soil loss of 167 t/ha were recorded with only one time plowing without

ridging. Highest yields were obtained by planting in planting holes (Tian Yinong *et al.*, 1995)

Other agronomic and soil conservation practices

Many experiments studied the effect of various combinations of agronomic practices to determine those that would result in high yields and low levels of soil erosion, while also being easy to install and maintain, and not too expensive or labor intensive. **Table 8** shows the effect of different methods of land preparation, weed control, intercrops and live-barriers, as well as that of fertilizer or manure application in a farmer's field with 40% slope in Mondomo, Cauca, Colombia. Among land preparation treatments, highest cassava yields were obtained by plowing with an oxen-drawn reversible plow, which is the standard practice in the area. However, the lowest level of erosion was obtained in plots without any land preparation, where cassava stakes were planted by pushing directly into the rather soft topsoil. Among weed control methods, highest yields were obtained when weeds were controlled by hoeing, but lowest levels of both cassava yield and erosion were obtained by using only a machete to cut off the weeds without disturbing the soil. Intercropping and various live-barriers slightly reduced cassava yields, but also reduced erosion, while the application of fertilizers markedly increased yields while also decreasing the soil loss by erosion (Howeler and Guzman, 1985).

Table 8. The effect of various soil and crop management treatments on cassava yields and soil erosion in a farmer's field with 40% slope in Mondomo, Cauca, Colombia.

		Cassava yield (t/ha)	Soil loss (t/ha)
A.	***Effect of methods of land preparation***		
	1. Planting holes	8.9	3.08
	2. Oxen with reversible plow	9.3	2.96
	3. Oxen with chisel plow	5.7	2.90
	4. Preparation by plow of 1 m strips alternated with 1 m wide strips without preparation	8.1	2.32
	5. Without preparation	7.9	1.59
B.	***Effect of methods of weed control***		
	1. With hoe	15.3	3.71
	2. With herbicides	11.3	3.55
	3. With machete	9.3	2.96
C.	***Effect of intercropping or live-barriers***		
	1. No intercrop or live-barriers	9.3	2.96
	2. Hedgerows of lemon grass	7.7	2.64
	3. Intercropped with beans	7.8	2.16
	4. Barriers of Imperial grass	7.0	1.88
	5. Barriers of *Brachiaria decumbens* grass	6.2	1.82
D.	***Effect of fertilizers***		
	1. Without fertilizers or lime	0.3	3.50
	2. With fertilizers: 500 kg/ha lime and 750 kg/ha 10-30-10	9.3	2.96

Many similar erosion control trials were conducted in Quilichao, Mondomo, Mondomito and Agua Blanca, in the cassava growing area of Cauca Department in Colombia from 1981 to 1986 (CIAT, 1985a; 1985b; 1988; Howeler, 1986; 1987). Subsequently, many other erosion control trials were conducted in Thailand, China, Philippines, Vietnam, Malaysia and Indonesia (Chan *et al*., 1994; Jantawat *et al*., 1991; 1992; 1994; Howeler, 1992; 1993; 1994; 1995; 1996; 1998; Howeler *et al*., 2001a; 2001b; Putthacharoen *et al*., 1998; Wargiono *et al*., 1995; Zhang Weite *et al*., 1998). Only the results of a few of these experiments are shown below.

Figure 5 shows the effect of cassava plant spacing, both in monoculture or when intercropped with upland rice and maize, on the total crop value (gross income) and on soil losses by erosion in Tamanbogo, Lampung Indonesia. At all plant spacings intercropping resulted in a slightly higher gross income than planting in monoculture, but planting cassava at 1x1 m resulted in a slightly higher income and lower erosion than planting at a wider row spacing, especially in case of monoculture. In case of intercropped cassava there was not much difference between the various spacing treatments, both in terms of gross income or erosion (Wargiono *et al*., 1995).

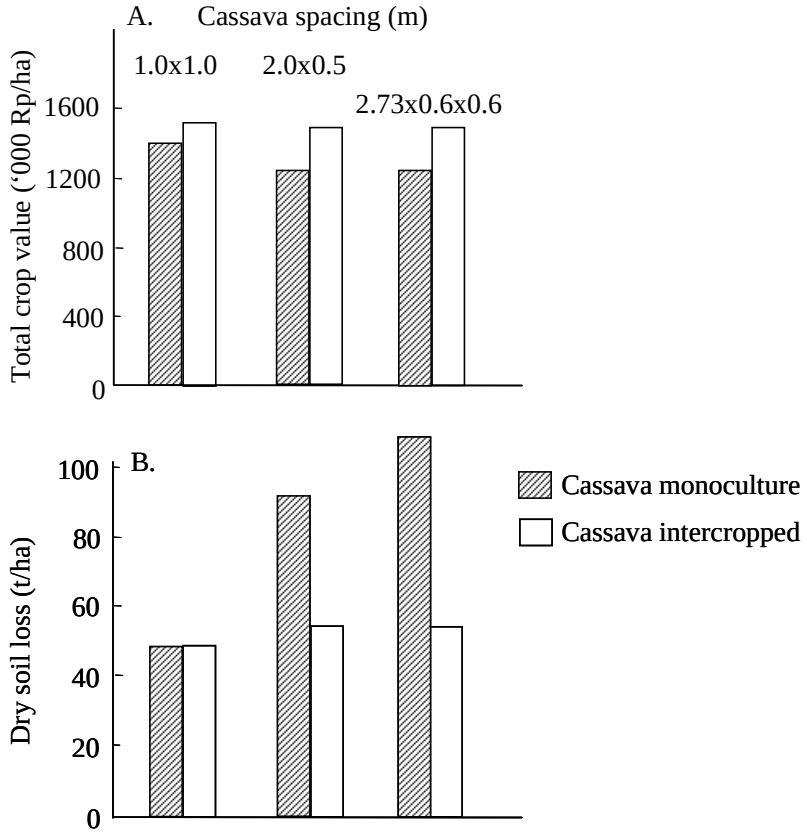

Figure 5. Effect of cassava plant spacing on total crop value (A) and on soil loss by erosion (B) when cassava was grown in monoculture or intercropped with upland rice and maize in Tamanbogo, Indonesia.
***Source**: Wargiono et al, 1995.*

Figure 6 shows the results of an erosion control trial conducted for five consecutive years at Jatikerto Experiment Station in Malang district of East Java, Indonesia. Cassava intercropped with maize was planted without hedgerows (check) or with hedgerows of *Pennisetum purpureum (*elephant grass), *Gliricidia sepium* or *Flemingia macrophylla.* The data on cassava yields and soil loss due to erosion in the treatments with the various hedgerows are expressed as a percentage of those in the check plot without hedgerows

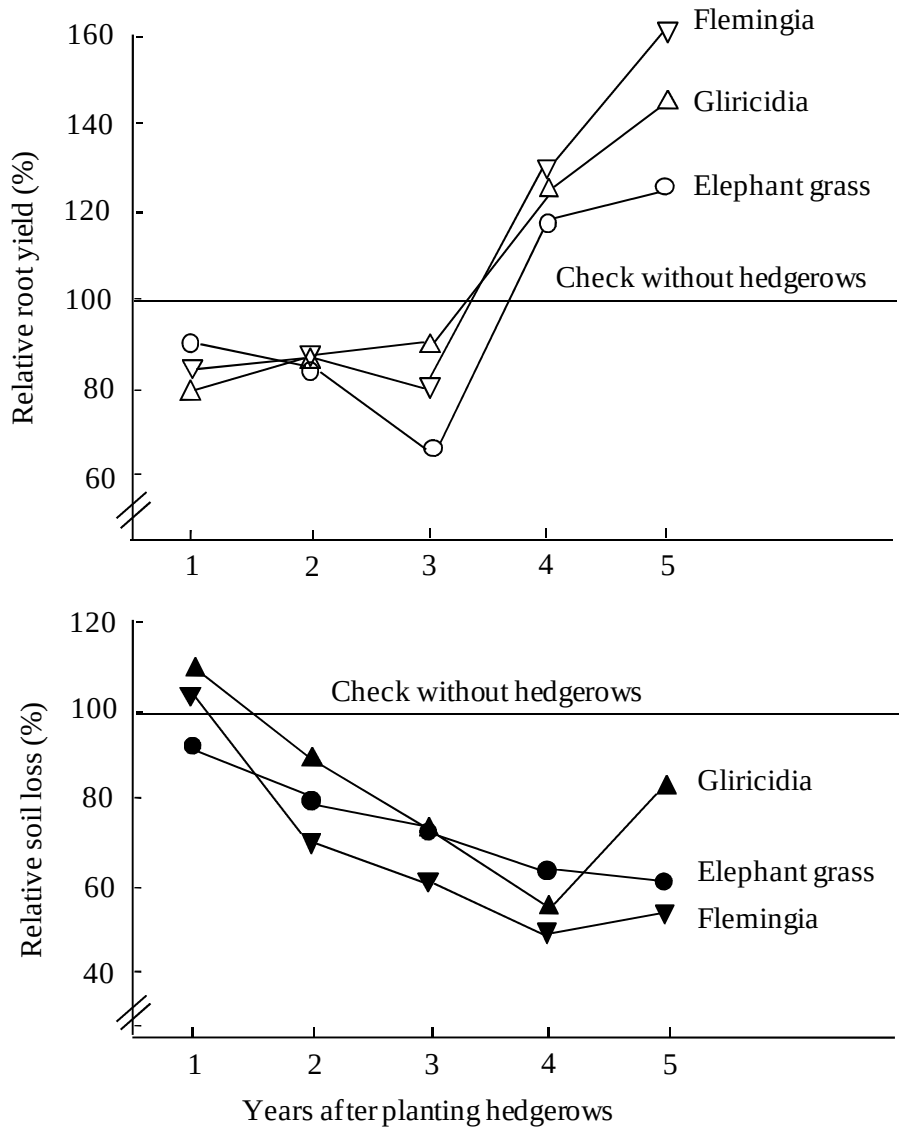

Figure 6. Trend in relative yield and relative soil loss due to erosion when cassava intercropped with maize was planted with contour hedgerows of elephant grass, Gliricidia sepium and Flemingia macrophylla during five consecutive years of cropping on 8% slope at Jatikerto, Malang, Indonesia from 1991/92 to 1996/97.

It is clear that initially the hedgerows decreased cassava yields by occupying land, but during the 4th and 5th year they caused a significant increase in yield, especially *Flemingia* and *Gliricidia* by supplying N to cassava in this extremely N-deficient soil. In the first year after establishment, the two leguminous tree species were also not effective in reducing erosion, but in subsequent years all three hedgerows became increasingly more effective, and during the 4th and 5th year had reduced soil losses to about 60% of those in the check plots without hedgerows (Wani Hadi Utomo, personal communication). Thus, planting cassava on slopes with contour hedgerows of leguminous tree species in an alley cropping system (see Chapter 18) can both increase yields and reduce erosion.

Similar results were also observed during 11 years of continuous cropping in South Vietnam. **Table 9** shows the results of an erosion control trial conducted at Hung Loc Agric. Research Center in Dongnai during the eleventh year of continuous cropping, using various intercrops and contour hedgerow species to reduce erosion. Highest cassava root yields were obtained by intercropping with peanut, but planting hedgerows of vetiver grass, *Leucaena leucocephala* or *Gliricidia sepium* markedly reduced erosion as compared to the check plot without hedgerows. Intercropping also reduced erosion but was not as effective as the hedgerows, especially those of vetiver grass. **Figure 7** shows that the effectiveness of the hedgerows in reducing erosion increased over time and that vetiver grass was consistently more effective than the other two leguminous tree species. The hedgerows also increased cassava yields about 10-20%. Similar results were obtained with hedgerows of vetiver grass or *Tephrosia candida*, which both reduced erosion to about 20% as compared to the check without hedgerows in FPR erosion control trials (Howeler, 2008).

Figure 8 shows the effect of various soil and crop management treatments on the accumulative soil losses due to erosion during a 10 month growth cycle of cassava in Sri Racha, Thailand. As in most other erosion control trials, soil losses were most serious during the first 4-5 months of growth, after which it decreased markedly because of complete canopy cover and the onset of the dry season. This and many other trials showed that soil losses were greatest in the absence of fertilizers, as this greatly delayed canopy formation. Least amount of soil loss was observed in the treatments of no tillage and with contour ridging. Intercropping with peanut also reduced erosion. However, this treatment resulted in the lowest cassava yield of 16.1 t/ha, slightly lower than those obtained without fertilizers (17.6) and no tillage (21.2). This compares with a yield of 27.1 t/ha for the treatment with complete tillage (2 plowing, 2 disking) plus contour ridging and fertilizer application.

Table 9. Effect of cropping systems and the planting of contour hedgerows on the yield of cassava and intercrops, on dry soil loss by erosion, and on gross and net income during the 11th consecutive year of cropping on 12% slope at Hung Loc Agric. Research Center in Thong Nhat district, Dong Nai, Vietnam in 2007/08.

Treatments[1)]	Dry soil loss (t/ha)	Root yield (t/ha)	Starch content (%)	Hedgerow yield (t/ha)	Gross income[2)]	Product. cost[3)]	Net income
					('000d/ha)		
1. C monoculture, no hedgerows	33.56	27.06	27.90	-	31,660	6,008	25,652
2. C+mungbean IC	28.84	32.60	28.03	2.19	38,142	8,108	30,034
3. C+peanut IC[4)]	22.46	34.58	29.43	3.76	41,595	8,108	33,487
4. C+vetiver hedgerows	10.03	30.45	28.73	10.10	35,626	7,008	28,618
5. C+*Leucaena* AC	16.50	30.09	30.00	10.21	35,205	7,008	28,197
6. C+*Gliricidia* AC	18.11	29.58	28.18	8.45	34,609	7,008	27,601

[1)] C = cassava; IC = intercrop; AC = alley crop

[2)] Prices: cassava dong 1,170/kg fresh roots
peanut 8,000/kg dry pods

[3)] Costs:

land preparation	900,000/ha
planting cassava	700,000/ha
planting intercrops	500,000/ha
seed intercrops	400,000/ha
weeding	2,200,000/ha
harvest or cutting of intercrops	1,200,000/ha
fertilizers (90:40:80 kg/ha)	1,983,000/ha
fertilizer application	225,000/ha

[4)] peanut yield: 142 kg dry pods/ha = dong 1,136,000

***Source**: Nguyen Huu Hy et al., 2010.*

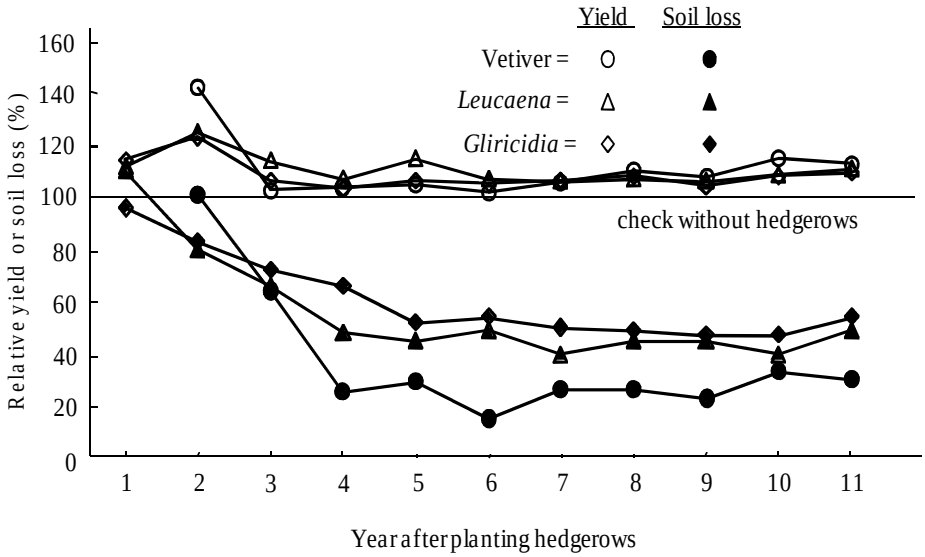

Figure 7. Trend in relative yield and relative soil loss by erosion when cassava was planted with contour hedgerows of vetiver grass, Leucaena leucocephala or Gliricidia sepium, in comparison with the check without hedgerows during eleven consecutive years in Hung Loc Agric. Research Center in South Vietnam from 1997/98 to 2008/09.
***Source**:Nguyen Huu Hy et al., 2010.*

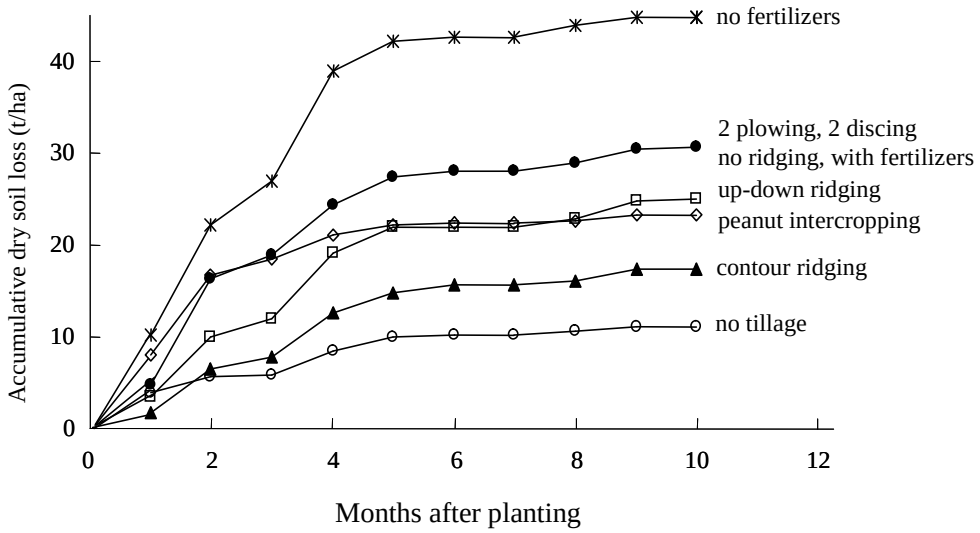

Figure 8. Effect of various soil/crop management practices on the accumulative dry soil loss by erosion in a farmers' field in Sri Racha, Thailand, during a ten month growth cycle of cassava in 1988/89.
***Source**: Howeler, 1992.*

Enhancing the Adoption of Soil Conservation Practices

From the many experiments conducted by researchers on experiment stations and on farm it is clear that there are many agronomic and soil conservation practices that can reduce soil losses by water erosion and even increase yields. This includes planting cassava at a closer plant spacing (at populations of >10,000 plants/ha), applying fertilizers or manures, planting contour hedgerows of certain grasses or leguminous tree species, contour plowing and ridging, applying mulch, and intercropping with peanut, melons or squash etc. However, most of these practices have certain advantages and disadvantages; some are very effective in reducing erosion, but also may reduce yields, and may be costly or laborious to install or maintain. **Table 10** shows the relative importance of the good and bad attributes of various soil conservation practices.

Since most soil conservation practices have advantages and disadvantages, trade-offs will need to be made. Those are best made by farmers themselves as they will greatly depend on the specific bio-physical as well as the socio-economic situation at each site. Thus, farmers were encouraged to conduct simple erosion control and various other types of trials on their own fields with guidance from researchers and extension workers. These were called Farmer Participatory Research (FPR) trials. From 1994 to 2004 farmers conducted a total of 1,621 FPR trials in 99 villages of Thailand, Vietnam, China and Indonesia, of which 378 erosion control trials. Some typical examples of these trials are shown in **Tables 11-13.**

During farmer field days at time of harvest, farmers from the village (participating and non-participating) and surrounding villages would visit each trial and evaluate and score each treatment according to their own criteria. Later in the day the average results of the each type of trial were presented for discussion with the farmers; this included estimates of the gross income, total production cost and net income for each treatment. Farmers were asked to raise hands to show how they had scored each treatment in order to calculate the farmers'preferences, as shown in the last columns of **Tables 11, 12 and 13.**

Table 10. Effect of various soil/crop management practices on erosion and yield, as well as on labor and monetary requirements and long-term benefits in cassava-based cropping systems.

Erosion control practices	Erosion control	Terrace formation	Effect on cassava yield	Labor requirement	Monetary cost	Long-term benefits	Main limitations
Minimum or zero tillage	++	-	-	+	- -	+	compaction, weeds
Mulching (carry-on)	++++	-	++	+++	+	++	mulch availability, transport
Mulching (in-situ production	+++	-	++	++	+	++	competition
Contour tillage	+++	+	+	+	+	++	
Contour ridging	+++	+	++	++	++	+	not suitable on steep slopes
Leguminous tree hedgerows	++	++	+	+++	+	+++ [1)]	delay in benefits
Cut-and-carry grass strips	++	++	- -	+++	+	+++ [1)]	competition, maintenance
Vetiver grass hedgerows	+++	+++	+	+	+	+++	
Natural grass strips	++	++	-	+	-	++	high maintenance costs
Cover cropping (live mulch)	++	-	- - -	+++	++	+	severe competition
Manure or fertilizer application	++++	-	+++	+	+++	+++	high cost
Intercropping	++	-	-	++	++	+++	labor intensive
Closer plant spacing	+++	-	+	+	+	++	

+ = effective, positive or high

- = not effective, negative or low

[1)] = value added in terms of animal feed, staking material or fuel wood.

Table 11. Effect of various crop management treatments on the yield of cassava and intercropped peanut a well as the gross and net income and soil loss due to erosion in a FPR erosion control trial conducted by six farmers in Kieu Tung village of Thanh Ba district, Phu Tho province, Vietnam in 1997 (3rd year).

Treatment[1)]	Slope (%)	Dry soil loss (t/ha)	Yield (t/ha) cassava	Yield (t/ha) peanut[1)]	Gross income[2)]	Product costs	Net income	Farmers ranking
					----(mil. dong/ha)-----			
1. C monocult., with fertilizer, no hedger.	40.5	106.1	19.17	-	9.58	3.72	5.86	6
2. C+P, no fertilizer, no hedgerows	45.0	103.9	13.08	0.70	10.04	5.13	4.91	5
3. C+P, with fertilizer, no hedgerows	42.7	64.8	19.23	0.97	14.47	5.95	8.52	-
4. C+P, with fertilizer, *Tephrosia* hedger.	39.7	40.1	14.67	0.85	11.58	5.95	5.63	3
5. C+P, with fertilizer, pineapple hedger.	32.2	32.2	19.39	0.97	14.55	5.95	8.60	2
6. C+P, with fertilizer, vetiver hedgerows	37.7	32.0	23.71	0.85	16.10	5.95	10.15	1
7. C monocult, with fert., *Tephrosia* hedger.	40.0	32.5	23.33	-	11.66	4.54	7.12	4

[1)] Fertilizers = 60 kg N + 40 P_2O_5, + 120 K_2O/ha; all plots received 10 t/ha pig manure

[2)] Prices: cassava (C) dong 500/kg fresh roots
peanut (P) 5000/kg dry pods

***Source**: Howeler, 2001.*

Table 12. Average results of two FPR erosion control trials conducted by farmers in Khook Anu village, Thep Sathit district of Chayaphum province, Thailand, in 2001/02.

Treatments	Dry soil loss (t/ha)	Yield (t/ha) Cassava	Yield (t/ha) Intercrop	Starch content (%)	Gross income	Product. costs[2)]	Net income	Farmers' preference (%)
					——(baht/ha)——			
1. farmer's practice	13.99	12.61	-	20.3	12,736	12,018	718	0
2. contour plowing	10.16	8.41	-	20.0	8,410	11,471	-3,061	100
3. up/down plowing	31.10	12.34	-	18.3	11,970	11,974	-4	0
4. mungbean intercrop	10.30	8.70	0.306	24.0	15,516	15,392	124	82
5. vetiver grass hedgerows	8.03	13.02	-	22.3	13,619	13,083	536	100
6. lemon grass hedgerows	4.53	15.94	-	21.0	16,259	13,550	2,709	0[3)]

[1)] Prices: cassava baht 1.20/ kg fresh roots at 30% starch
mungbean 20/ kg dry grain

[2)] Cost of production without harvest baht 10,000/ha
harvest + transport 160/tonne
contour plowing 125/ha extra
C+mungbean intercrop 14,000/ha
hedgerow planting + maintenance 1,000/ha

[3)] Although lemon grass hedgerows produced the highest net income, farmers do not like this practice because lemon grass does not tolerate drought and it is difficult to sell in large quantities.

***Source:** Howeler, 2008.*

Table 13. Average results of five FPR erosion control trials conducted by farmers in Tien Phong and Dac Son villages of Pho Yen district, Thai Nguyen province, Vietnam, in 1997.

Treatments[1)]	Dry soil loss[1)] (t/ha)	Yield (t/ha)		Gross income[3)]	Production costs[4)] (mil. dong/ha)	Net income	Farmers' preference (%)
		cassava	peanut[2)]				
1. Farmer's practice	7.73	11.77	-	5.89	4.05	1.84	0
2. C+P, contour ridges	5.39	17.47	0.36	10.54	5.64	4.90	0
3. C+P, contour ridges, vetiver hedgerows	3.94	19.05	0.37	11.38	5.92	5.46	67
4. C+P, contour ridges, *Tephrosia* hedgerows	3.02	19.00	0.39	11.45	5.92	5.53	83
5. C+P, contour ridges, *Tephrosia*+vetiver hedgerows	2.73	17.92	0.41	11.01	5.92	5.09	3

[1)]Farmer's practice: cassava monoculture, 11.4 t/ha of FYM+68 kg N+20 P_2O_5+50 K_2O/ha; all other plots received 10 t/ha of FYM+80 kg N + 40 P_2O_5 + 80 K_2O/ha

[2)]dry pods

[3)]Prices: cassava: dong 600/kg fresh roots
peanut: 5,000/kg dry pods

[4)]Costs FYM: dong 100/kg
urea (45%N): 2,500/kg
SSP (17% P_2O_5): 1,000/kg
KCl (60%K_2O): 2,500/kg
peanut seed: 6,000/kg; use 50 kg/ha
labor: 7,500/manday
1 US $ = 11.000 dong

***Source**: Nguyen The Dang et al., 2001*.

The average effect of the various soil and crop management practices on cassava yields and on dry soil loss due to erosion were calculated as a percentage of a check treatment without the practice for all erosion control experiments and FPR trials conducted in Thailand and Vietnam. The results are shown in **Tables 14** and **15**. In both countries contour hedgerows of vetiver or *Paspalum atratum*, were most effective in controlling erosion, while in Vietnam hedgerows of *Tephrosia candida, Flemingia macrophylla* and pineapple were also very effective. In Thailand these hedgerows slightly reduced yields because they take up some space in the field, but in Vietnam they actually increased cassava yields 10-15%. Planting cassava at a closer spacing was also quite effective in reducing erosion in Thailand but not in Vietnam; in both countries closer spacing increased cassava yields. Hedgerows of leguminous tree species like *Leucaena* or *Gliricidia* were intermediately effective in controlling erosion and increased cassava yields only in long-term trials in Vietnam. Application of fertilizers was one of the most effective ways to increase cassava yields and markedly reduce soil losses by erosion, especially in Vietnam. Intercropping with peanut, melon or sweet corn did not reduce erosion and decreased cassava yields in Thailand (although they may have increased total income), while intercropping with peanut was intermediately effective in reducing erosion and slightly increased cassava yields in Vietnam.

Table 14. Effect of various soil conservation practices on the average[1)] relative cassava yield and dry soil loss due to erosion as determined from soil erosion control experiments, FPR demonstration plots and FPR trials conducted in Thailand from 1994 to 2003.

	Soil conservation practices[2)]	Relative cassava yield (%)	Relative dry soil loss (%)
1.	With fertilizers; no hedgerows, no ridging, no intercrop (check)	100	100
2.	With fertilizers; vetiver grass hedgerows, no ridging, no intercrop**	90 (25)	58 (25)
3.	With fertilizers; lemon grass hedgerows, no ridging, no intercrop**	110 (14)	67 (15)
4.	With fertilizers; sugarcane for chewing hedgerows, no intercrop	99 (12)	111 (14)
5.	With fertilizers; *Paspalum atratum* hedgerows, no intercrop**	88 (7)	53 (7)
6.	With fertilizers; *Panicum maximum* hedgerows, no intercrop	73 (3)	107 (4)
7.	With fertilizers; *Brachiaria brizantha* hedgerows, no intercrop*	68 (3)	78 (2)
8.	With fertilizers; *Brachiaria ruziziensis* hedgerows, no intercrop*	80 (2)	56 (2)
9.	With fertilizers; elephant grass hedgerows, no intercrop	36 (2)	81 (2)
10.	With fertilizers; *Leucaena leucocephala* hedgerows, no intercrop*	66 (2)	56 (2)
11.	With fertilizers; *Gliricidia sepium* hedgerows, no intercrop*	65 (2)	48 (2)
12.	With fertilizers; *Crotalaria juncea* hedgerows, no intercrop	75 (2)	89 (2)
13.	With fertilizers; pigeon pea hedgerows, no intercrop	75 (2)	90 (2)
14.	With fertilizers; contour ridging, no hedgerows, no intercrop**	108 (17)	69 (17)
15.	With fertilizers; up-and-down ridging, no hedgerows, no intercrop	104 (20)	124 (20)
16.	With fertilizers; closer spacing, no hedgerows, no intercrop**	116 (10)	88 (11)
17.	With fertilizers; C+peanut intercrop	72 (11)	102 (12)
18.	With fertilizers; C+pumpkin or squash intercrop	90 (13)	109 (15)
19.	With fertilizers; C+sweet corn intercrop	97 (11)	110 (14)
20.	With fertilizers; C+mungbean intercrop*	74 (4)	41 (4)
21.	No fertilizers; no hedgerows, no or up/down ridging	96 (9)	240 (10)

[1)] number in parenthesis indicates the number of experiments/trials from which the average values were calculated.
[2)] C = Cassava
** = most promising soil conservation practices; * = promising soil conservation practices
***Source**: Howeler, 2001*.

At the end of the project in 2004 an impact assessment was conducted by an outside consultant to determine which practices were most widely adopted by farmers in Thailand and Vietnam. This was done by focus group discussions with farmers that previously had participated in the FPR trials and training courses, as well as farmers living in nearby villages that had not participated directly in the project. Farmers were also asked to fill in census forms to indicate which practices they had adopted and what their cassava yields were before and after the project. Results, shown in **Table 16**, indicate that among the participating farmers 53% in Thailand and 31% in Vietnam were using contour ridging to control erosion. Among non-participating farmers this was only 22 and 29%, respectively, resulting in an overall adoption of about 30% in both countries.

Concerning the adoption of contour hedgerows, it is clear that these were adopted mainly by those farmers that had actively participated in the project. Interestingly, the great majority of farmers in Thailand preferred the planting of vetiver grass, while those in North Vietnam preferred *Tephrosia candida* and in South Vietnam *Paspalum atratum*. Other types of hedgerows, like lemon grass or pineapple, while being quite effective in reducing erosion, were seldom adopted. This clearly indicates that farmers select those practices that fit best into their existing farming practices and are most suitable for their own particular conditions

Table 15. Effect of various soil conservation practices on the average[1)] relative cassava yield and dry soil loss due to erosion as determined from soil erosion control experiments, FPR demonstration plots and FPR trials conducted in Vietnam from 1993 to 2003.

		Rel. cassava yield (%)		Rel. dry soil loss (%)	
	Soil conservation-practices[2)]	Cassava monoculture	Cassava + peanut	Cassava monoculture	Cassava + peanut
1.	With fertilizers; no hedgerows (check)	100	-	100	-
2.	With fertilizers; vetiver grass hedgerows**	113 (17)	115 (23)	48 (16)	51 (23)
3.	With fertilizers; *Tephrosia candida* hedgerows**	110 (17)	105 (23)	49 (16)	64 (23)
4.	With fertilizers; *Flemingia macrophylla* hedgerows*	103 (3)	109 (4)	51 (3)	62 (3)
5.	With fertilizers; *Paspalum atratum* hedgerows**	112 (17)	-	50 (17)	-
6.	With fertilizers; *Leucaena leucocephala* hedgerows*	110 (11)	-	69 (11)	-
7.	With fertilizers; *Gliricidia sepium* hedgerows*	107 (11)	-	71 (11)	-
8.	With fertilizers; pineapple hedgerows*	100 (8)	103 (9)	48 (8)	44 (9)
9.	With fertilizers; vetiver+*Tephrosia* hedgerows	-	102 (7)	-	62 (7)
10.	With fertilizers; contour ridging; no hedgerows*	106 (7)	-	70 (7)	-
11.	With fertilizers; closer spacing, no hedgerows	122 (5)	-	103 (5)	-
12.	With fertilizers; peanut intercrop; no hedgerows*	106 (11)	100	81 (11)	100
13.	With fertilizers; maize intercrop; no hedgerows	69 (3)	-	21 (3)	-
14.	No fertilizers; no hedgerows	32 (4)	92 (15)	137 (4)	202 (12)

[1)] number in parenthesis indicates the number of experiments/trials from which the average values were calculated.

[2)] IC = intercrop, HR = hedgerows

** = most promising soil conservation practices; * = promising soil conservation practices

***Source**: Howeler, 2001*.

Table 16. Extent of adoption (percent of households) of soil conservation technologies by participating and non-participating farmers in the Nippon Foundation cassava project in Thailand and Vietnam[1)].

	Participants			Non-participants		
Soil conservation practices	Thailand	Vietnam	Overall	Thailand	Vietnam	Overall
- contour ridging	53.0	31.3	40.9	22.0	28.9	25.0
- hedgerows - vetiver grass	61.5	11.6	33.7	9.6	3.7	7.0
- *Tephrosia candida*	0	32.7	18.2	0	6.9	3.0
- *Paspalum atratum*	0.9	11.6	6.8	0	2.0	0.9
- pineapple	0	2.7	1.5	0	0.8	0.4
- sugarcane	1.7	0	0.8	0.6	0	0.4
- other hedgerows	3.4	7.5	5.7	0.3	1.6	0.9
- no soil conservation	20.5	29.3	25.4	70.8	59.3	65.8

[1)] Data are based on census forms filled by 417 households in Thailand and 350 in Vietnam, of which 109 and 126 had been participants of the project, respectively.

***Source:** Dalton et al., 2007*.

In Thailand vetiver grass is popular because it is recommended by the King and young plants are readily available, usually free of charge. This is not the case in Vietnam, so obtaining vegetative planting material in large quantities is more difficult. Farmers in the north prefer *Tephrosia candida* because it grows well in the cooler climate and as a leguminous species is expected to improve the soil. In the south farmers prefer *Paspalum*

atratum because it provides feed for cattle and buffaloes. Thus, in order to achieve adoption of soil conservation practices, researchers should not promote a single technology because it happens to be effective in experiments, but they should let farmers conduct their own soil erosion control trials, and let farmers select the practices that are most suitable for their own conditions.

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CHAPTER 21

FARMER PARTICIPATION IN RESEARCH AND EXTENSION: THE KEY TO ACHIEVING ADOPTION OF MORE SUSTAINABLE CASSAVA PRODUCTION PRACTICES IN ASIA[1] [2]

Reinhardt H. Howeler[3]

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the third most important food crop (after rice and maize) grown in southeast Asia, and is used for human consumption, animal feed and for industrial purposes. It is usually grown by smallholders in upland areas with poor soils and low or unpredictable rainfall. In some countries the crop is grown on steep slopes, but in others it is grown mainly on gentle slopes; in both cases, soil erosion can be serious. Moreover, cassava farmers seldom apply adequate amounts of fertilizers or manures to replace the nutrients removed in the harvested products. Thus, both erosion and nutrient extraction can result in a decline in soil fertility and a gradual degradation of the soil resource.

The fact that farmers do not apply sufficient fertilizers and do not use soil conservation practices when the crop is grown on slopes is more a socio-economic rather than a technical problem. Research has shown many ways to maintain or improve soil fertility and reduce erosion, but farmers usually consider these practices too costly or requiring too much labor. To overcome these obstacles to adoption it is necessary to develop simple practices that are suitable for the local situation and that provide short-term benefits to the farmer as well as long-term benefits in terms of resource conservation. Being highly site specific these practices can best be developed by the farmers themselves, on their own fields, in collaboration with research and extension personnel.

Thus, a project was initiated, with financial support from the Nippon Foundation in Tokyo, Japan, to develop a farmer participatory methodology for the development and dissemination of more sustainable production practices in cassava-based cropping systems, that will benefit a large number of poor farmers in the uplands of Asia.

1. FIRST PHASE (1994-1999)

The first phase of the project was conducted in four countries, i.e. China, Indonesia, Thailand and Vietnam. The project was coordinated by CIAT and implemented in collaboration with research and extension organizations in each of the four countries. During an initial training course on farmer participatory research (FPR) methodologies, each country designed a work plan to implement the project. The general steps in the process, from diagnosing the problem to adoption of suitable solutions, are shown in **Figure 1**. The outstanding feature of this approach is that farmers participate in every step and make all important decisions. Researchers and extensionists show farmers various options, they facilitate the research and extension activities, but do not make any recommendations or promote any particular technologies.

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[1] This chapter is a modified version of Howeler *et al*., 2007.

[2] For color photos see pages 774-778.

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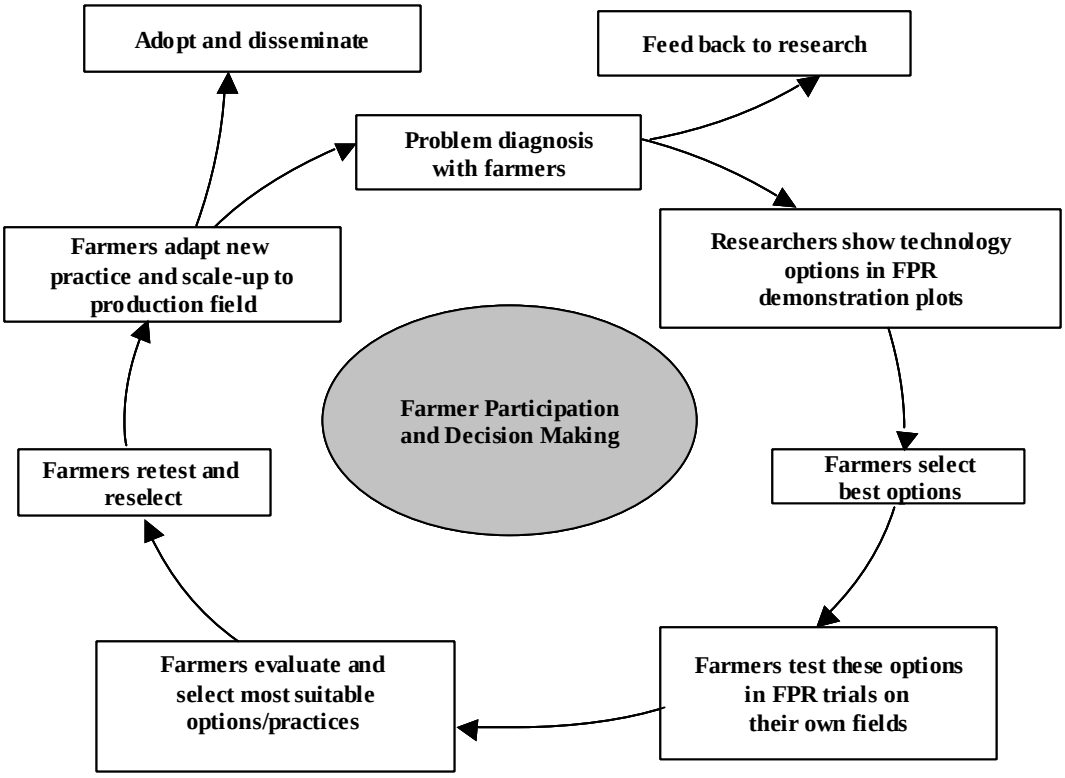


Figure 1. Farmer participatory model used for the development of sustainable cassava-based cropping systems in Asia

a. Pilot site selection

Suitable pilot sites were pre-selected in areas where cassava is an important crop, where it is grown on slopes and erosion is a serious problem. Detailed information obtained through Rapid Rural Appraisals (RRA) in each site have been reported by Nguyen The Dang *et al*. (1998), Utomo *et al*. (1998), Vongkasem *et al*. (1998) and Zhang Weite *et al*. (1998). **Table 1** is an example of information obtained from RRAs conducted in Vietnam, while **Table 2** shows a summary of information obtained from RRAs' conducted in several pilot sites in four countries. The detailed information from each site can serve as baseline data to monitor progress and evaluate the impact of newly adopted technologies. After conducting the RRAs, the most suitable pilot sites (villages or subdistricts) were selected to work with farmers in the development and dissemination of new varieties and production practices.

b. Demonstration plots

Each year demonstration plots were laid out on an experiment station or a farmer's field to show the effect of many alternative treatments on yield, income and soil erosion.

Table 1. Cropping systems, varieties and agronomic practices, as determined from RRAs conducted in four FPR pilot sites in Vietnam in 1996/97.

Province	Hoa Binh	Phu Tho	Thai Nguyen	
District	Luong Son	Thanh Ba	Pho Yen	
Village		Phuong Linh		
Hamlet	Dong Rang	Kieu Tung	Tien Phong	Dac Son
Cropping system[1)]				
-upland	tea C+T C monoculture peanut, maize	C monoculture C+P tea, peanut maize	C+P or C+B or 2 yr C rotated with 2 yr fallow sweet potato	C monocult. or C-P rotation or C-B, C-SP sweet potato
Varieties				
-rice	CR 203, hybrids from China	DT 10, DT 13, CR 203	DT 10, DT 13 CR 203	CR 203 DT 10, DT 13
-cassava	Vinh Phu, local	Vinh Phu, local	Vinh Phu	Vinh Phu
Cassava practices				
-planting time	early March	early March	Feb/March	Feb/March
-harvest time	Nov/Dec	Nov/Dec	Nov/Dec	Nov/Dec
-plant spacing (cm)	100x80	80x80; 80x60	100x50	100x50
-planting method	horiz./inclined	horizontal	horiz./inclined	horizontal
-land preparation	buffalo/cattle	by hand/cattle	buffalo	buffalo
-weeding	2 times	2 times	2 times	2 times
-fertilization	basal	basal+side[2)]	basal+side[3)]	basal+side[4)]
-ridging	mounding	flat	flat	flat
-mulching	rice straw	peanut residues	peanut residues	peanut residues
-root chipping	hand chipper	knife	small grater	small grater
-drying	3-5 days	3-5 days	2-4 days	2-4 days
Fertilization				
cassava				
-pig manure (t/ha)	5	5	3-5	8-11
-urea (kg/ha)	0	50-135	83	83-110
-SSP (18% P_2O_5) (kg/ha)	50-100	0	140	0-280
-KCl (kg/ha)	0	0	55	0-280
rice				
-pig/buffalo manure (t/ha)	5	0	-	-
-urea (kg/ha)	120-150	80	-	-
Yield (t/ha)				
-cassava	11-12	8-15	8.5	8.7
-rice (per crop)	3.3-4.2	4.2	3.0-3.1	2.7-3.0
-taro	1.9-2.2	-	-	-
-sweet potato	-	-	8.0	3.3
-peanut	0.8-1.2	0.5-1.1	1.4	1.3
pigs (kg live weight/year)	100-120	-	-	-

[1)] C=cassava, P=peanut, B=black bean, T=taro, M=maize
C+P=cassava and peanut intercropped; C-P=cassava and peanut in rotation

[2)] urea at 2 MAP

[3)] urea when 5-10 cm tall; NPK+FYM when 20 cm tall

[4)] NPK when 30 cm tall; hill up

Table 2. Characteristics of eight pilot sites for the Farmer Participatory Research (FPR) trials in Asia in 1994/95.

	Thailand		Vietnam			China	Indonesia	
	Soeng Saang	Wang Nam Yen	Pho Yen	Thanh Ba	Luong Son	Kongba	Malang	Blitar
Mean temp. (°C)	26-28	26-28	16-29	25-28	16-29	17-27	25-27	25-27
Rainfall (mm)	950	1400	2000	~1800	~1700	~1800	>2000	~1500
Rainy season	Apr-Oct	Apr-Nov	Apr-Oct	Apr-Nov	May-Oct	May-Oct	Oct-Aug	Oct-June
Slope (%)	5-10	10-20	3-10	30-40	10-40	10-30	20-30	10-30
Soil	± fertile loamy Paleustult	± fertile clayey Haplustult	infertile sandy loam Ultisol	very infertile clayey Ultisol	± fertile clayey Paleustult	± fertile sandy cl.l. Paleudult	infertile clay loam Mollisol	infertile clay loam Alfisol
Main crops	cassava rice fruit trees	maize soybean cassava	rice sweet pot. maize	rice cassava tea	rice cassava taro	rubber cassava sugarcane	cassava maize rice	maize cassava rice
Cropping system[1)]	C monocrop	C monocrop	C monocrop	C monocrop	C+T	C monocrop	C+M	C+M
Cassava yield (t/ha)	17	17	10	4-6	15-20	20-21	12	11
Farm size (ha)	4-24	3-22	0.7-1.1	0.2-1.5	0.5-1.5	2.7-3.3	0.2-0.5	0.3-0.6
Cassava (ha/hh)	2.4-3.2	1.6-9.6	0.07-0.1	0.15-0.2	0.3-0.5	2.0-2.7	0.1-0.2	0.1-0.2

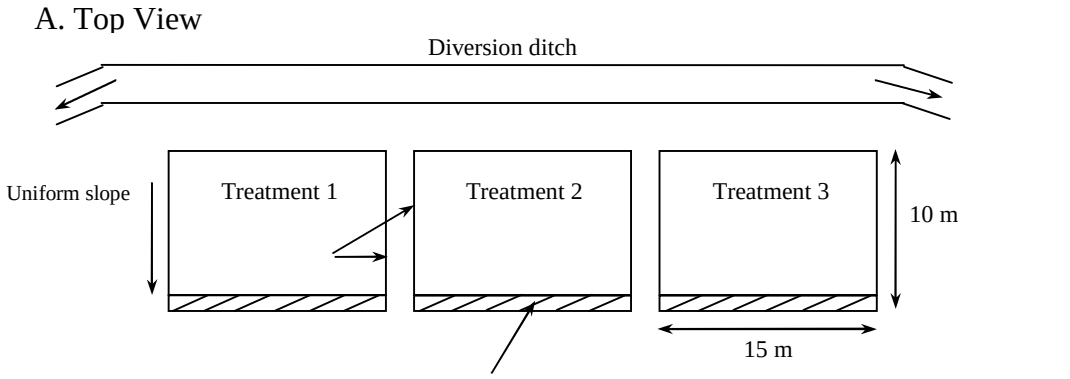
[1)] C = cassava, T = taro, M = maize

Farmers from the selected pilot sites visiting the trial were asked to discuss and score the usefulness of each treatment. From this range of many options farmers usually selected 3-4 treatments that they considered most useful for their own conditions. **Table 3** shows that farmers from different sites have different priorities and thus rank options quite differently. Some farmers then volunteered to test these treatments in FPR trials on their own fields.

In both the demonstration plots and FPR erosion control trials on farmers' fields, a simple methodology was used to measure soil loss due to erosion in each treatment. Plots were laid out carefully and exactly along the contour on a uniform slope. It is important that runoff water does not enter the plots either from above or from the sides. Along the lower side of each plot a ditch was dug and covered with plastic (**Figure 2**); small holes in de plastic allowed runoff water to seep away while eroded sediments remained on the plastic. These sediments were collected and weighed monthly or at least 2-3 times during the cropping cycle. After correcting for moisture content, the amount of dry soil loss per hectare was calculated for each treatment. This simple methodology gives both a visual as well as a quantitative indication of the effectiveness of the various practices in controlling erosion (Howeler, 2001; 2002).

Table 3. Ranking of conservation farming practices selected from demonstration plots as most useful by cassava farmers from several pilot sites in Asia in 1995/96.

	Thailand		Vietnam		China	Indonesia	
	Soeng Saang	Wang Nam Yen	Pho Yen	Thanh Hoa	Baisha	Blitar	Dampit
Farm yard manure (FYM)				2			
Medium NPK	5						
High NPK					2		
FYM + NPK				1			
Cassava residues incorporated			5				
Reduced tillage	4						
Contour ridging		2					
Up-and-down ridging					5		
Maize intercropping	2					1	1
Peanut intercropping		5			4		2
Mungbean intercropping					3		
Black bean intercrop			1	4			
Tephrosia green manure			3	5			
Tephrosia candida hedgerows			4				
Gliricidia sepium hedgerows						2	4
Vetiver grass barriers	1	1	2	3			
Brachiaria ruziziensis barriers	3	4					
Elephant grass barriers						3	3
Lemon grass barriers		3					
Stylosanthes barriers					1		



[1)]Plot border of sheet metal, wood or soil ridge to prevent water, entering or leaving plots.
[2)]polyethylene or PVC plastic sheet with small holes in bottom to catch eroded soil sediments but allow run-off water to seep away. Sediments are collected and weighed once a month.

Figure 2. Experimental lay-out of simple trials to determine the effect of soil/crop management practices on soil erosion.

c. FPR trials

The FPR trials not only involved soil conservation practices, but also new varieties, intercropping systems and fertilization, with the objective of developing a combination of practices that would increase farmers' income, reduce erosion and improve soil fertility. The FPR trials usually had 4-6 treatments, with one treatment representing the farmer's traditional variety or practice. Plot size varied from a minimum of 30 m^2 to a maximum of 100 m^2. Treatments were not replicated, but wherever possible, farmers within one village

conducting the same type of trial were encouraged to use the same treatments, so that each trial could be considered a replication and results could be averaged over those replications. This increased the confidence in the reliability of the results.

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During the first phase of the project, farmers in the four countries conducted a total of 177 FPR erosion control trials, 157 variety trials, 98 fertilizer trials and 35 intercropping trials, for a total of 467 trials. At time of harvest, field days were organized in each site to harvest the various trials by the participating farmers and their neighbors. The yields of cassava and intercrops, the dry soil loss due to erosion, as well as the gross income, production costs and net income were calculated for each treatment and presented to the farmers. Farmers and extension workers from the area discussed the results and then indicated their preferences for a particular treatment or production practice by raising their hands.

After one or more years of testing in small plots, farmers quickly identified the best varieties and production practices for their particular conditions and started using those on larger areas of their production fields (Howeler, 2002).

Table 4 shows a typical example of an FPR erosion control trial conducted by six farmers having adjacent plots on about 40% slope. Contour hedgerows of vetiver grass, *Tephrosia candida* or pineapple reduced erosion to about 30% of that in the check plot, while intercropping with peanut and planting vetiver hedgerows also markedly increased net income. Farmers clearly preferred those treatments that were most effective in both increasing net income and reducing soil erosion, such as hedgerows of vetiver grass or pineapple. Results of many other FPR trials have been reported by Nguyen The Dang *et al*. (2001), Huang Jie *et al*. (2001), Utomo *et al*. (2001) and Vongkasem *et al.* (2001).

c. Scaling up and adaptation

After having selected the most promising varieties and production practices from FPR trials, farmers generally like to test some of these on small areas of their production fields, making adaptations if necessary. Some practices may look promising on small plots, but are rejected as impractical when applied on larger areas; this may be due to lack of sufficient planting material (like vetiver grass) or lack of markets for selling the products (like pumpkin or lemon grass). Also, to be effective, hedgerows need to follow the contour rather precisely; otherwise they can cause serious gully erosion by channeling runoff water to the lowest spot. Contour hedgerows also force farmers to plow along the contour, which is more difficult and more costly; moreover it makes planting in neat straight lines, using tight strings as a guide, impossible. Thus, there are very practical reasons why farmers may be reluctant to adopt some of these soil conservation practices. **Table 5** shows the particular technologies that farmers had adopted in the four countries at the end of the first phase of the project.

Table 4. Effect of various crop management treatments on the yield of cassava and intercropped peanut as well as the gross and net income and soil loss due to erosion in a FPR erosion control trial conducted by six farmers in Kieu Tung village of Thanh Ba district, Phu Tho province, Vietnam in 1997 (3rd year).

Treatment[1)]	Slope (%)	Dry soil loss (t/ha)	Yield (t/ha) cassava[2)]	Yield (t/ha) peanut[2)]	Gross income[3)] ---------	Product. costs (mil. dong/ha)-------	Net income	Farmers ranking
1. C monocult., with fertilizer, no hedgerows	40.5	106.1	19.17		9.58	3.72	5.86	6
2. C+P, no fertilizer, no hedgerows	45.0	103.9	13.08	0.70	10.04	5.13	4.91	5
3. C+P, with fertilizer, no hedgerows	42.7	64.8	19.23	0.97	14.47	5.95	8.52	-
4. C+P, with fertilizer, *Tephrosia* hedgerows	39.7	40.1	14.67	0.85	11.58	5.95	5.63	3
5. C+P, with fertilizer, pineapple hedgerows	32.2	32.2	19.39	0.97	14.55	5.95	8.60	2
6. C+P, with fertilizer, vetiver hedgerows	37.7	32.0	23.71	0.85	16.10	5.95	10.15	1
7. C monocult, with fert., *Tephrosia* hedgerows	40.0	32.5	23.33		11.66	4.54	?	4

[1)] Fertilizers = 60 kg N + 40 P_2O_5, + 120 K_2O/ha; all plots received 10 t/ha pig manure
T_1=farmer's traditional practice

[2)] Cassava: fresh roots; peanut: dry pods

[3)] Prices: cassava (C) dong 500/kg fresh roots
peanut (P) 5000/kg dry pods
1US$ = approx. 13.000 dong

Table 5. Technological components selected and adopted by participating farmers from their FPR trials conducted from 1994 to 1998 in four countries in Asia.

Technology	China	Indonesia	Thailand	Vietnam
Varieties	SC 8013***[1)] SC 8634* ZM 9247* OMR35-70-7*	Faroka*** 15/10* OMM90-6-72*	Kasetsart 50*** Rayong 5*** Rayong 90**	KM 60*** KM 94* KM 95-3*** SM1717-12*
Fertilizer practices	15-5-20+Zn+ chicken manure 300 kg/ha*	FYM 10 t/ha (TP)+ 90 N+36 P_2O_5+ 100 K_2O**	15-15-15 156 kg/ha***	FYM 10 t/ha (TP)+ 80 N+40 P_2O_5+ 80 K_2O**
Intercropping	monoculture (TP) C+peanut	C+maize (TP)	monoculture (TP) C+pumpkin* C+mungbean*	monoculture (TP) C+taro (TP) C+peanut***
Soil conservation	sugarcane barrier*** vetiver barrier*	*Gliricidia* barrier** *Leucaena* barrier* contour ridging**	vetiver barrier*** sugarcane barrier**	*Tephrosia* barrier*** vetiver barrier* pineapple barrier*

[1)] * = some adoption; ** = considerable adoption; *** = widespread adoption;
TP = traditional practice; FYM = farm-yard manure

2. SECOND PHASE (1999-2004): FARMER PARTICIPATORY RESEARCH (FPR) AND EXTENSION (FPE)

The second phase of the project was conducted in collaboration with five institutions in Thailand, six in Vietnam and three in China (**Table 6**). During the second phase the emphasis shifted from the development and use of farmer participatory research (FPR) methodologies to farmer participatory extension (FPE) in order to reach more farmers and achieve more widespread adoption.

During both the first and second phase of the project some collaborative research continued on-station in order to solve problems identified at the farm level, or to develop better technologies that farmers could later test on their own fields.

Table 6. Partner institutions collaborating in the second phase of the Nippon Foundation cassava project in Asia.

1. Research and extension organizations in Thailand
-Department of Agriculture (DOA)
-Department of Agricultural Extension (DOAE)
-Land Development Department (LDD)
-Kasetsart University (KU)
-The Thai Tapioca Development Institute (TTDI)
2. Research and extension organizations in Vietnam
-Thai Nguyen University of Agriculture and Forestry (TNUAF)
-National Institute for Soils and Fertilizers (NISF)
-Vietnam Agricultural Science Institute (VASI)
-Hue University of Agriculture and Forestry (HUAF)
-Institute of Agricultural Sciences of South Vietnam (IAS)
-Tu Duc University of Agriculture and Forestry (TDUAF)
3. Research and extension organizations in China
-Chinese Academy for Tropical Agricultural Sciences (CATAS)
-Guangxi Subtropical Crops Research Institute (GSCRI)
-Honghe Animal Husbandry Station of Yunnan

Once farmers had selected certain practices and wanted to adopt those on their fields, the project staff tried to help them; for instance, in setting out contour lines to plant hedgerows for erosion control, or to obtain seed or vegetative planting material of the selected hedgerow species, intercrops or new cassava varieties.

Since the objective of the second phase was to achieve widespread adoption of more sustainable production practices by as large a number of farmers as possible, it was necessary to markedly expand the number of pilot sites and to develop farmer participatory extension (FPE) methodologies to disseminate the selected practices and varieties to many more farmers.

a. Farmer participatory research (FPR)

Implementing the project in collaboration with many different institutions in China, Thailand and Vietnam (**Table 6**), and with generous financial support from the Nippon Foundation, it was possible to expand the number of pilot sites each year. In 2001 the project had been working in about 50 sites, and this further increased to 99 sites by the end of the project in 2004 (**Figure 3**). Once the benefits of the new technologies became clear, the number of sites increased automatically as neighboring villages also wanted to participate in order to increase their yields and income.

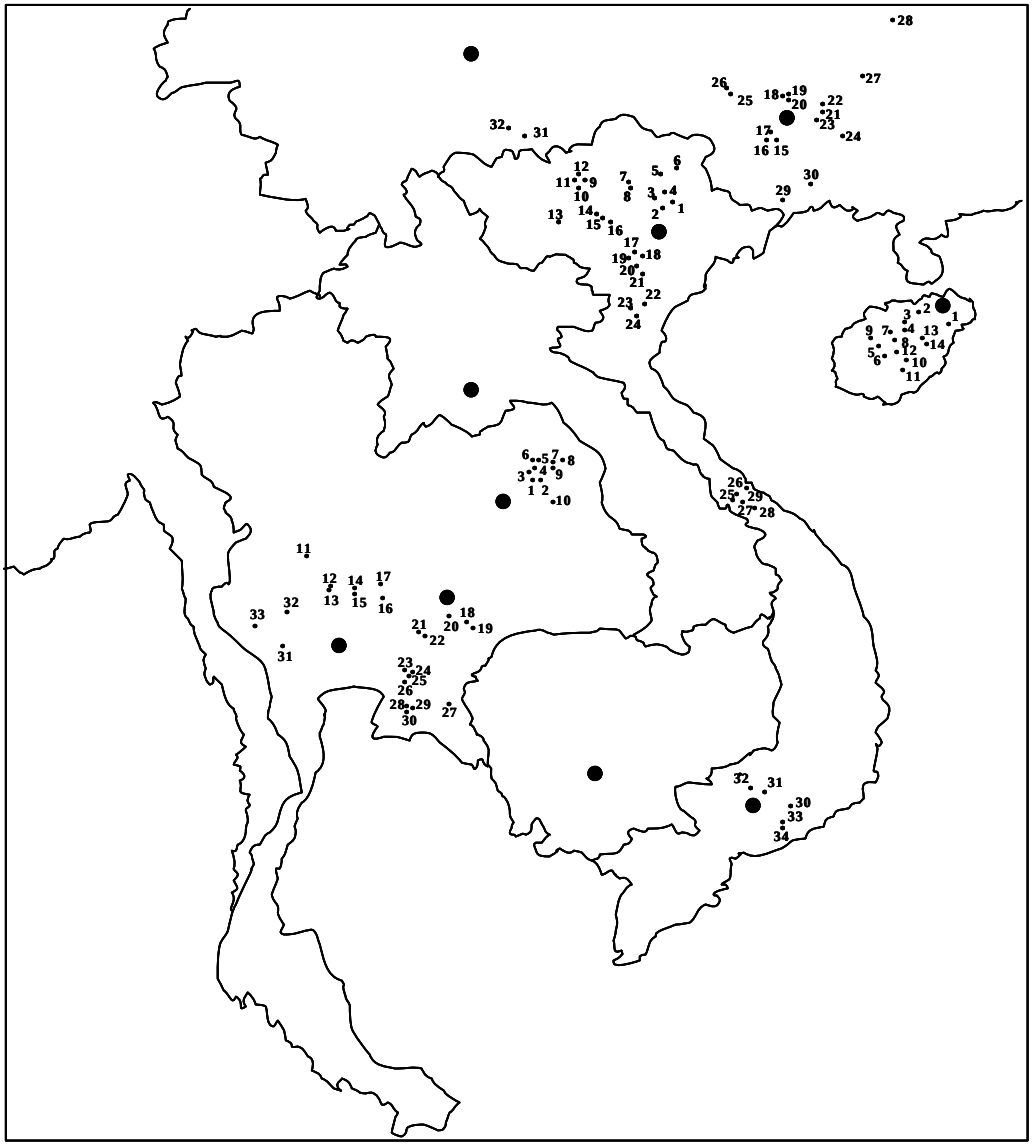

Figure 3. Location of FPR pilot sites in China, Thailand and Vietnam in the Nippon Foundation cassava project in 2003/04.

Whenever the project extended to a "new" site, the process outlined above was re-initiated, i.e. an RRA was conducted, interested farmers visited demonstration plots and/or made a cross-visit to an already established site; farmers conducted FPR trials, discussed results and eventually adopted those varieties or practices they had selected as most suitable for their own conditions. **Table 7** shows the number and types of FPR trials conducted in China, Thailand and Vietnam during the second phase of the project. While initially farmers were mainly interested in testing new varieties, fertilization, intercropping and erosion control practices, during the later part of the project they also wanted to test the use of organic or green manures, weed control, plant spacing and even leaf production and pig feeding. During the five years of the second phase of the project a total of 1,154 FPR trials were conducted by farmers on their own fields. **Tables 8** to **12** are just a few examples of the various types of FPR trials conducted by farmers in different sites in Thailand and Vietnam.

Table 7. Number of FPR trials conducted in the 2d phase of the Nippon Foundation cassava project in China, Thailand and Vietnam.

Country	Type of FPR trial	1999	2000	2001	2002	2003	**Total**
China	Varieties	9	9	20	69	20	**127**
	Erosion control	3	5	8	17	-	**33**
	Fertilization	-	-	-	4	-	**4**
	Intercropping	-	-	-	9	-	**9**
	Pig feeding	-	-	-	59	-	**59**
		12	**14**	**28**	**158**	**20**	**232**
Thailand	Varieties	11	16	16	19	25	**87**
	Erosion control	14	10	6	-	11	**41**
	Chemical fertilizers	16	6	23	17	17	**79**
	Chem.+org fertilizers	-	-	10	11	11	**32**
	Green manures	-	-	13	11	15	**39**
	Weed control	-	-	17	5	10	**32**
	Plant spacing	-	-	3	-	2	**5**
	Intercropping	-	-	16	7	-	**23**
		41	**32**	**104**	**70**	**91**	**338**
Vietnam	Varieties	12	31	36	47	35	**161**
	Erosion control	16	28	29	30	23	**126**
	Fertilization	1	23	36	24	24	**108**
	Intercropping	-	14	32	31	26	**103**
	Weed control	-	3	-	-	3	**6**
	Plant spacing	-	1	7	19	8	**35**
	Leaf production	-	-	2	2	1	**5**
	Pig feeding	-	-	11	16	13	**40**
		29	**100**	**153**	**169**	**133**	**584**
Total		**82**	**146**	**285**	**397**	**244**	**1,154**

Table 8. Results of an FPR variety trial conducted by a farmer in Am Thang commune, Son Duong district, Tuyen Quang, Vietnam in 2002.

Treatments[1)]	Cassava yield (t/ha)	Gross income	Product. costs	Net income	B/C	Farmers' preference[2)] (%)
		----------('000 dong/ha)----------				
1. Vinh Phu (local)	20.70	10,350	4,330	6,020	2.39	7.9
2. La Tre (SC205) (local)	21.40	10,700	4,330	6,370	2.47	10.5
3. KM60	29.20	14,600	4,330	10,270	3.37	21.0
4. KM94	37.50	18,750	4,330	14,420	4.33	94.7
5. KM95-3	32.80	16,400	4,330	12,070	3.79	26.3
6. KM98-7	25.40	12,700	4,330	8,370	2.93	10.5

[1)] fertilized with 1,100 kg/ha of 7-4-7 fertilizers = 1.43 mil. dong/ha
[2)] out of 38 farmers

Table 9. Average results of three FPR erosion control trials conducted by farmers in Suoi Rao and Son Binh villages, Chau Duc district, Baria-Vungtau, Vietnam in 2003/04.

Treatments	Dry soil loss (t/ha)	Cassava yield (t/ha)	Maize+ hedgerow yield (t/ha)	Gross income[1)]	Product. costs[2)]	Net income	Farmers' preference (%)
				——('000 dong/ha)——			
1. cassava monoculture, no hedgerows	77.12	26.34	-	10,536	6,079	4,457	20
2. C+ pineapple hedgerows	11.65	27.02	-	10,808	6,279	4,529	0
3. C+ *Paspalum atratum* hedgerows	12.18	30.13	11.40	12,052	6,279	5,773	65
4. C+ vetiver grass hedgerows	9.94	28.33	8.84	11,332	6,279	5,053	15
5. C+ maize intercrop	14.30	17.86	3.25	10,394	7,969	2,425	0

[1)] Prices:	cassava	dong	400/kg fresh roots
	maize		1,000/kg dry grain
[2)] Costs:	labor		20,000/manday
	cassava fertilizers		1,279,000 dong/ha
	maize fertilizers		550,000 dong/ha
	cassava stakes		500,000 dong/ha
	maize seed		440,000 dong/ha
	labor for cassava without HR (210 md/ha)	=	4.2 mil. dong/ha
	labor for maize (40 md/ha)	=	0.8 mil. dong/ha
	labor for fertilizer application (5 md/ha)	=	0.1 mil. dong/ha
	labor for hedgerow cutting/maintenance	=	0.2 mil. dong/ha

Table 10. Results of an FPR fertilizer and manure trial conducted in Khut Dook village, Baan Kaw, Daan Khun Thot, Nakhon Ratchasima, Thailand in 2002/03.

Treatments[1)]	Root yield (t/ha)	Starch content (%)	Gross income[2)]	Fertilizer cost[3)] ('000 B/ha)	Production costs[3)]	Net income
1. No fertilizers or manure	18.75	25.0	21.56	0	10.87	10.69
2. Chicken manure+rice hulls, 400 kg/rai	30.42	26.2	34.98	2.50	17.15	17.83
3. Pelleted chicken manure, 100 kg/rai	26.70	21.1	30.71	2.00	15.39	15.32
4. 15-7-18 fertilizer, 50 kg/rai	29.68	24.1	34.13	2.66	16.73	17.40
5. 13-13-21 fertilizer, 50 kg/rai	32.22	27.4	37.05	3.13	17.89	19.16
6. 16-20-0 fertilizer, 50 kg/rai	26.08	25.9	29.99	2.50	15.61	14.38
7. 15-15-15 fertilizer, 50 kg/rai	30.36	26.9	34.91	2.81	17.07	17.84

[1)] 1ha = 6.25 rai

[2)] Prices: cassava baht 1.15 /ton irrespective of starch content

[3)] Costs:
- chicken manure 1.0 /kg
- pelleted chicken manure 3.20 /kg
- 15-7-18 8.50 /kg
- 13-13-21 10.0 /kg
- 16-20-0 3.0 /kg
- 15-15-15 9.0 /kg
- harvest + transport roots 270 /ton
- cassava production without fertilizer or harvest 12,757/ha

Table 11. Average results of four FPR intercropping trials conducted by farmers in Tran Phu commune, Chuong My district, Ha Tay, Vietnam in 2003.

Treatments	Cassava yield (t/ha)	Intercrop yield (t/ha)	Gross income[1)]	Seed costs[2)] ('000d/ha)	Production costs[2)]	Net income
1. Cassava monoculture	24.54	-	9,816	0	5,460	4,356
2. C+1 row peanut	21.93	1.187	14,707	480	8,115	6,592
3. C+2 rows peanut	22.52	2.000	19,008	960	8,595	10,413
4. C+2 rows mungbean	21.42	0	8,568	2000	9,635	-1,067
5. C+2 rows soybean	21.28	0.162	9,322	800	8,435	887

[1)] Prices:
- cassava: dong 400/kg fresh roots
- peanut: 5,000/kg dry pods
- soybean 5,000/kg dry seed

[2)] Costs:
- labor: dong 15,000/manday
- NPK fertilizers: = 0.86 mil. dong/ha
- peanut seed (80 kg/ha): 12,000 /kg = 0.96 mil. dong/ha for 2 rows
- mungbean seed (80 kg/ha): 25,000 /kg = 2.00 mil. dong/ha for 2 rows
- soybean seed (80 kg/ha) 10,000 /kg = 0.80 mil. dong/ha for 2 rows
- labor for cassava monoculture without fertilizers = 4.5 mil. dong/ha (300 md/ha)
- labor for cassava intercropping without fertilizers = 6.675 mil.dong/ha (445 md/ha)
- labor for cassava fertilizer application = 0.10 mil. dong/ha

Table 12. Average results of five FPR pig feeding trials on adding ensiled cassava leaves to the diet, conducted by farmers in Huong Ha commune, A Luoi, Thua Thien-Hue, Vietnam in 2001/02.

Treatments	No. of pigs	Life weight (kg) initial	Life weight (kg) 3 months	LWG[1] (g/day)	FCR[2] (kg DM/kg gain)	Feed cost[5] (VND/kg gain)
Control diet[3]	6	24.30	52.50	313.3	4.83	10,745
Control + 13% ECL[4]	6	26.92	57.75	342.5	4.36	7,862
F test						*

[1] LWG = live weight gain
[2] FCR = feed conversion ratio
[3] Control diet of rice bran, ensiled cassava roots (32% as DM), fish meal and sweet potato vines
[4] 13% ensiled cassava leaves replaced part of fish meal, all SP vines; cassava leaves had been ensiled with 20% fresh grated cassava roots
[5] Prices:

rice bran	dong	2,000/kg
fish meal		6,000/kg
cassava roots		320/kg
fresh SP vines		400/kg
cassava leaves		3,000/20 kg

b. Farmer participatory extension (FPE)

The following farmer participatory extension methods were found to be very effective in raising farmers' interest in soil conservation, in disseminating information about improved varieties and cultural practices, and in enhancing adoption of soil conserving practices:

i. Cross-visits

Farmers from new sites were usually taken to visit older sites that had already conducted FPR trials and had adopted some soil conserving technologies. These cross-visits, in which farmers from the older site could explain their reasons for adopting new technologies was a very effective way of farmer-to-farmer extension. After these cross-visits, farmers in some new sites decided to adopt some technologies immediately, while others decided to conduct FPR trials in their own fields first. In both cases, the "FPR teams" of the various collaborating institutions, together with provincial, district or subdistrict extension staff, helped farmers to establish the trials, or they provided seed or planting materials required for the adoption of the new technologies.

ii. Field days

At time of harvest, field days were organized at the site in order to harvest the trials and discuss the results. Farmers from neighboring villages were usually invited to participate in these field days, to evaluate each treatment in the various trials and to discuss the *pros* and *cons* of the various practices or varieties tested.

In a few cases, large field days were also organized with participation of hundreds of neighboring farmers, school children, local and high-level officials, as well as representatives of the press and TV. The broadcasting or reporting about these events also helped to disseminate the information about suitable technologies. During the field days

farmers explained the results of their own FPR trials to the other visiting farmers, while extension pamphlets and booklets about the farmer-selected technologies were distributed.

iii. Training

Research and extension staff involved in the project had previously participated in Training-of-Trainers courses in FPR methodologies, including practical training sessions with farmers in some of the pilot sites. While some participants were initially skeptical, most course participants became very enthusiastic about this new approach once they started working more closely with farmers.

In addition, 2-3 key farmers from each site together with their local extension agent were invited to participate in FPR training courses. The objective was to learn about the various FPR methodologies, the basics of doing experiments as well as the implementation of commonly selected technologies, such as setting out contour lines or the planting, maintenance and multiplication of hedgerow species. By spending several days together in these courses, the farmers and extensionist got to know each other well, and they were encouraged to form a local "FPR team" to help other farmers in their community conduct FPR trials or adopt the new technologies.

iv. Community-based self-help groups

Realizing that effective soil conservation practices, such as planting of contour hedgerows, can best be done as a group, farmers from some sites decided to form their own "soil conservation group". These community-based self-help groups are similar to "Land Care units", that have been very effective in promoting soil conservation in the Philippines and Australia. Subsequently, the Dept. of Agric. Extension in Thailand encouraged farmers to set up these groups as a way of organizing themselves, to conduct FPR trials, to implement the selected practices, and to manage a rotating credit fund, from which members of the group can borrow money for production inputs. Thus, by 2003, a total of 21 "Cassava Development Villages" had been set up in the pilot sites in Thailand. Each group needed to have at least 40 members, elect five officers to lead the group, and establish their own bylaws about membership requirements, election of officers, use of the rotating fund, etc. The formation of these groups helped to decide on collective action and to strengthen the community, while people gained confidence and the group became more self-reliant. When necessary, the group could request help from local or national extension services, obtain information about certain production problems, or get planting material of vetiver grass or other species for hedgerows or green manures. Some groups started their own vetiver grass nurseries to have planting material available when needed.

Effect of New Technologies on Cassava Yield and Soil Loss by Erosion

Farmers are interested in testing new technologies only if those technologies promise substantial economic benefits over their traditional practices. Thus, strategic and applied research need to continue to produce and select still better varieties, better production practices and new utilization options. As such, some collaborative research in the area of agronomy and soil management continued.

1. Long-term fertility maintenance:

Long-term NPK trials were continued in four locations, one each in north and south Vietnam, one in Hainan island of China and one in southern Sumatra of Indonesia. **Figure**

4 shows the effect of annual applications of various levels of N, P, and K on the yield and starch content of two varieties during the 14th year of continuous cropping in Hung Loc Agricultural Research Center in South Vietnam. It is clear that, similar to most other locations, the main yield response was to the application of K, while there were minor responses to the application of N and P and mainly in the higher yielding variety SM 937-26. The combined application of 160 kg N, 80 P_2O_5 and 160 K_2O/ha increased yields from about 12 to 30 t/ha.

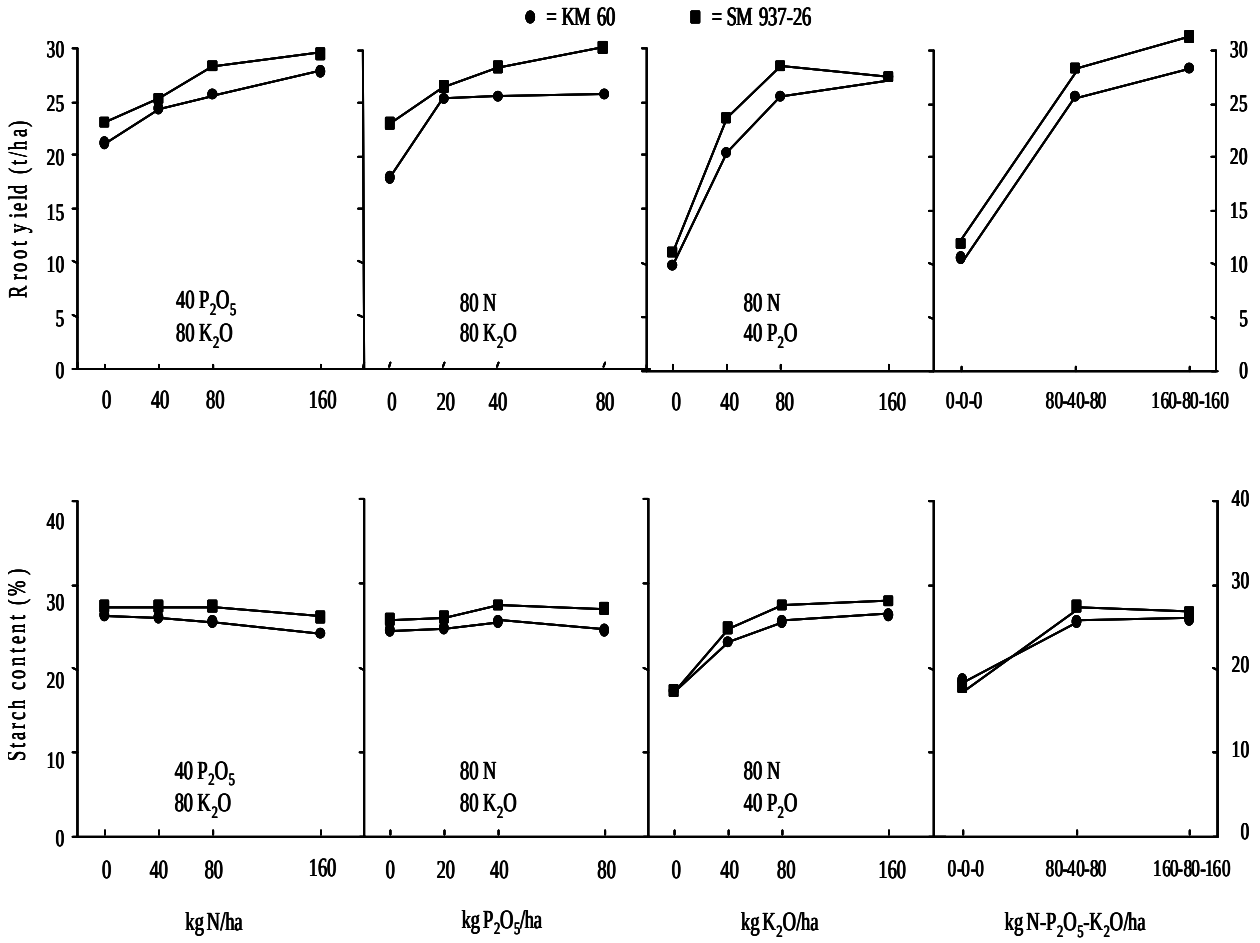

Figure 4. Effect of annual application of various levels of N, P and K on the root yield and starch content of two cassava varieties grown at Hung Loc Agric. Research Center in Thong Nhat, Dong Nai, Vietnam in 2003/04 (14th year).

2. Effect of various soil conservation practices on cassava yield and soil loss by erosion

Table 13 shows the average effect of various soil conservation practices on relative cassava yields and dry soil loss by erosion from numerous trials conducted in Thailand from 1994 to 2003. Closer plant spacing, lemon grass hedgerows and contour ridging were the most effective in both increasing yields and decreasing erosion. Most other contour hedgerow species, including vetiver grass, decreased cassava yields – mostly by reducing the area available for cropping and by competition with nearby cassava – but were very effective in reducing soil loss by erosion Most effective in reducing erosion were vetiver grass, *Paspalum atratum* and lemon grass, which reduced erosion by 33 to 47%. Intercropping was usually not effective in reducing erosion, while up-and-down ridging and especially the lack of fertilization markedly increased erosion. Similar results were obtained in Vietnam where hedgerows of vetiver grass, *Tephrosia candida* and *Paspalum atratum* all decreased erosion by about 50%, while also increasing cassava yields 10-13% (Howeler *et al*., 2004b; 2005).

Table 13. Effect of various soil conservation practices on the average[1)] relative cassava yield and dry soil loss due to erosion as determined from soil erosion control experiments, FPR demonstration plots and FPR trials conducted in Thailand from 1994 to 2003.

	Soil conservation practices[2)]	Relative cassava yield (%)	Relative dry soil loss (%)
1.	With fertilizers; no hedgerows, no ridging, no intercrop (check)	100	100
2.	With fertilizers; vetiver grass hedgerows, no ridging, no intercrop**	90 (25)	58 (25)
3.	With fertilizers; lemon grass hedgerows, no ridging, no intercrop**	110 (14)	67 (15)
4.	With fertilizers; sugarcane for chewing hedgerows, no intercrop	99 (12)	111 (14)
5.	With fertilizers; *Paspalum atratum* hedgerows, no intercrop**	88 (7)	53 (7)
6.	With fertilizers; *Panicum maximum* hedgerows, no intercrop	73 (3)	107 (4)
7.	With fertilizers; *Brachiaria brizantha* hedgerows, no intercrop*	68 (3)	78 (2)
8.	With fertilizers; *Brachiaria ruziziensis* hedgerows, no intercrop*	80 (2)	56 (2)
9.	With fertilizers; elephant grass hedgerows, no intercrop	36 (2)	81 (2)
10.	With fertilizers; contour ridging, no hedgerows, no intercrop**	108 (17)	69 (17)
11.	With fertilizers; up-and-down ridging, no hedgerows, no intercrop	104 (20)	124 (20)
12.	With fertilizers; closer spacing, no hedgerows, no intercrop**	116 (10)	88 (11)
13.	With fertilizers; C+peanut intercrop	72 (11)	102 (12)
14.	With fertilizers; C+pumpkin or squash intercrop	90 (13)	109 (15)
15.	With fertilizers; C+sweetcorn intercrop	97 (11)	110 (14)
16.	With fertilizers; C+mungbean intercrop*	74 (4)	41 (4)
17.	No fertilizers; no hedgerows, no or up/down ridging	96 (9)	240 (10)

[1)] number in parenthesis indicates the number of experiments/trials from which the average values were calculated.

[2)] C = Cassava

** = most promising soil conservation practices; * = promising soil conservation practices

The beneficial effects of contour hedgerows tend to increase markedly over time. **Figure 5** shows the long-term effect of contour hedgerows of vetiver grass and *Tephrosia candida* on relative cassava yields and soil loss as compared to the check plot without hedgerows; data are average values from three FPR erosion control trials conducted by farmers for nine consecutive years in north Vietnam. Although the results are rather variable, there is a clear trend that the two types of hedgerows caused a 20-40% increase in cassava yields and reduced soil losses by erosion to 20-40% of those in the check plots without hedgerows. Vetiver grass tended to become more effective in reducing soil losses than *Tephrosia*, firstly because the grass is more effective in filtering out suspended soil sediments, and secondly because *Tephrosia* hedgerows need to be replanted every 3-4 years, in contrast to vetiver grass which is a more or less permanent barrier. While farmers claim that *Tephrosia* improves the fertility of the soil more so than vetiver grass, the data show that vetiver grass increased cassava yields more than *Tephrosia*, probably by reducing losses of top soil and fertilizers and improving water infiltration and soil moisture content.

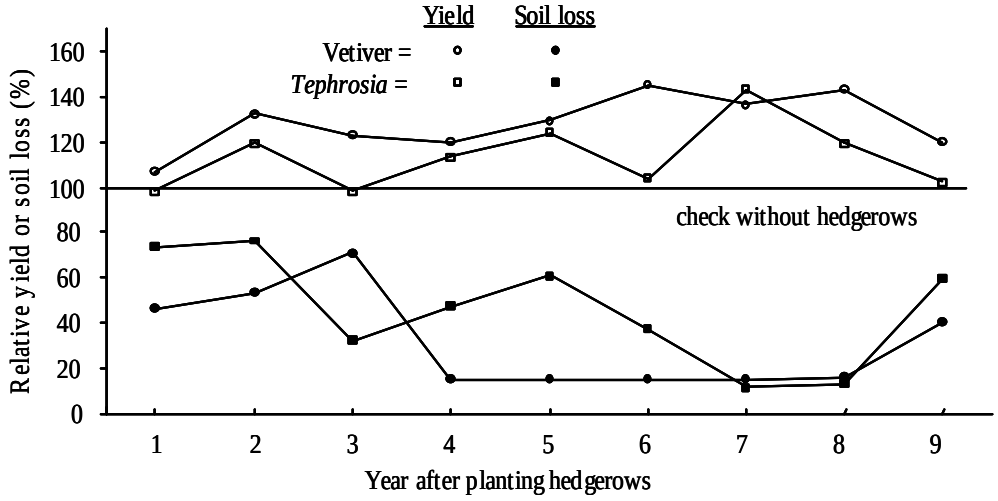

Figure 5. Trend in relative yield and relative soil loss by erosion when cassava was planted with contour hedgerows of vetiver grass or Tephrosia candida during nine consecutive years of cassava cropping. Data are average values from one FPR erosion control trial in Kieu Tung and two trials in Dong Rang in North Vietnam from 1995 to 2003.

ADAPTATION

After 2-3 years of testing of various options in FPR trials, slowly narrowing down the number of best options, farmers started to adopt some of the tested varieties or practices on their bigger production fields. In some cases they made adaptations so as to make the practices more suitable on a larger scale. For instance, in Thailand farmers planted contour hedgerows of vetiver grass on their fields, but left enough space between hedgerows (usually 30-40 m) to facilitate land preparation by tractor. In some cases, especially in Vietnam, farmers planted hedgerows on plot borders rather than along contour lines. This reduces the amount of land occupied by hedgerows, but also reduces their effectiveness in controlling erosion.

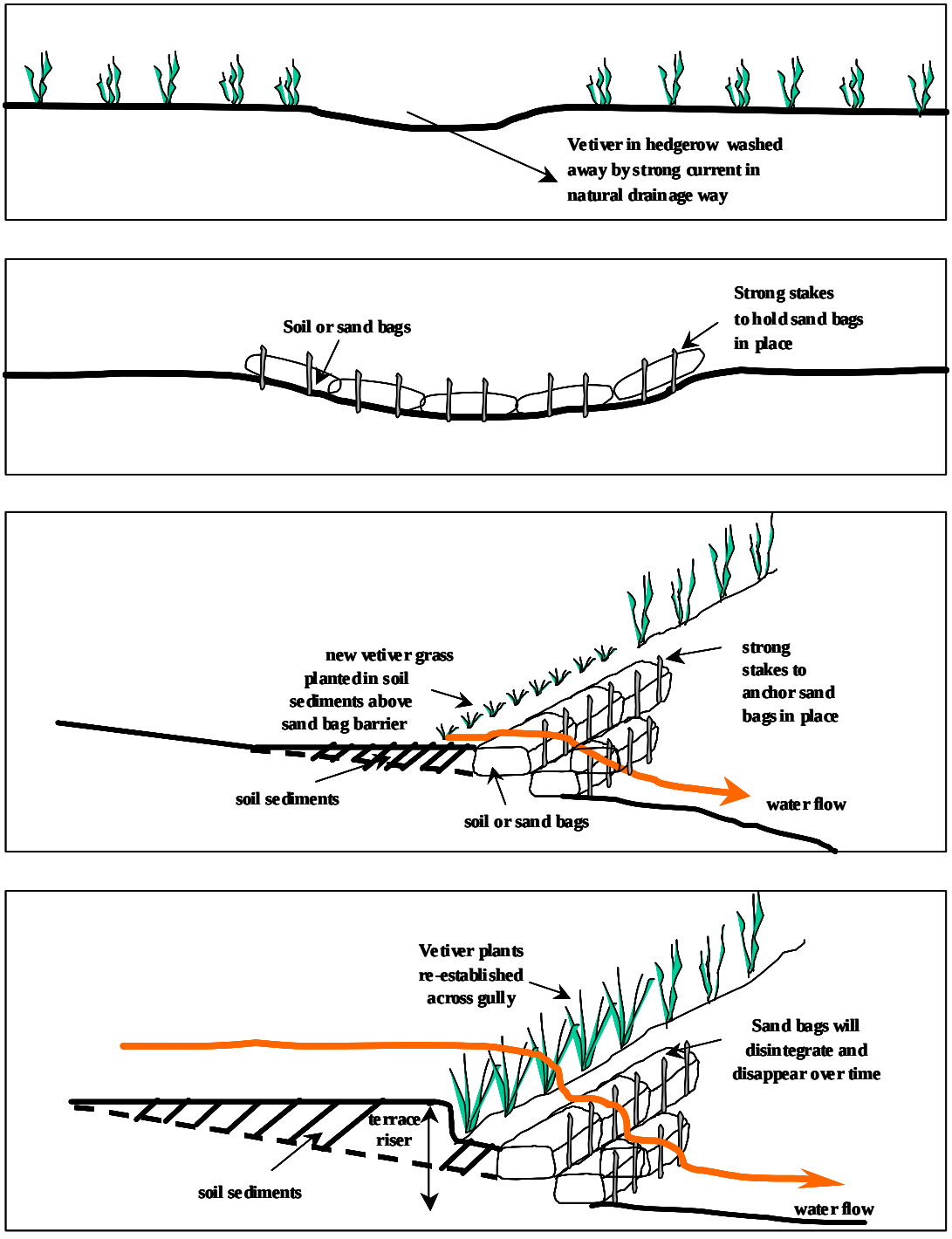

Figure 6. Simple and effective way to repair gullies by placing soil bags across the gull and planting vetiver grass in the soil sediments accumulating above the barrier.

While contour hedgerows of vetiver grass are usually the most effective in reducing soil losses by erosion in experiments and FPR trials conducted in small plots on a uniform slope, when this practice is scaled up to a larger production field the results are often disappointing. In areas of rolling terrain large amounts of runoff water may accumulate and run down-slope in natural drainage ways. The force of the water is likely to wash out vetiver grass recently planted along the contour across the drainage way, and this may result in serious gully erosion. Attempts to repair these gullies by placing sand bags or other obstacles across them have usually failed as these obstacles too are washed away. Over the past few years farmers and project staff have experimented informally with ways to reduce the speed of water in these gullies. They found that it is most effective to place a row of soil-filled plastic fertilizer bags across the gully in line but slightly below the washed out vetiver hedgerow. The bags need to be secured in place by pounding bamboo stakes into the soil behind them (**Figure 6**). Once eroded soil is deposited in the gully above the soil bags, vetiver grass can be planted in this moist and fertile sediment. When the vetiver grass is well-established across the gully and in line with the rest of the hedgerow, this will further slow the speed of runoff water resulting in further deposition of sediments in the gully above the vetiver hedgerow. This allows weeds to reestablish in the gully bottom protecting the gully from further erosion. With the next plowing along the contours, parallel to the hedgerows, the gully will generally be filled up again with soil, while the hedgerow prevents further gully formation (**Figure 6**). In some sites in Thailand, terraces of up to a meter height were formed within two years by the placing of soil bags and planting of vetiver hedgerows across the gully. This local adaptation of the traditional contour hedgerow system markedly increased its effectiveness under real field conditions.

ADOPTION

After conducting their own FPR trials, or after a cross-visit to another village where those trials were being conducted, farmers often decided to adopt one or more technologies on their production fields with the hope of increasing yields or income and protecting the soil from further degradation.

In Thailand, practically all of the cassava area is now planted with new varieties and about 75% of farmers apply some chemical fertilizers (TTDI, 2000), although usually not enough nor in the right proportion. As a result of the FPR fertilizer trials, farmers started to apply more K, while the official fertilizer recommendation for cassava was changed from an NPK ratio of 1:1:1 to 2:1:2. After trying various ways of controlling erosion, most farmers selected the planting of vetiver grass contour hedgerows as the most suitable. By the end of 2003, about 1,038 farmers had planted a total of 1.63 million vetiver plants, corresponding to about 145 km of hedgerows (Howeler *et al*., 2003; 2004a; 2004b; 2005; Vongkasem *et al*., 2003).

In Aug 2002 a participatory monitoring and evaluation (PM&E) was conducted in four pilot sites in Thailand where the project had been initiated at least four years earlier. Using focus group discussions and participatory evaluation methodologies, data were collected on the extent of adoption of the various technologies and the reasons for adoption or non-adoption. **Table 14** shows that new varieties had been adopted in 100% of the cassava growing areas in all four sites. Application of chemical fertilizers varied from 79-100%, vetiver hedgerows were planted in 22-55% of the cassava area, green manures in 0-50% and intercropping was not adopted at all, mainly due to lack of labor for managing intercrops. **Table 15** shows in more detail how the various technologies changed over the years, mainly as a result of conducting FPR trials on their own fields. While in most sites

some new varieties (Rayong 3, Rayong 60, Rayong 90) were already planted before the project started, the mix of new varieties changed over the years as higher yielding varieties were released, tested and adopted. The data also indicate how the use of chemical fertilizers not only increased over time, but also changed from the standard 15-15-15 to various formulations high in N and K and low in P.

Table 14. Extent of adoption[1)] of various cassava technology components in four pilot sites in Thailand in 2002 as a result of the Nippon Foundation project.

Technology component	Baan Khlong Ruam Sra Kaew		Thaa Chiwit Mai Chachoengsao		Sapphongphoot Nakhon Ratchasima		Huay Suea Ten Kalasin	
	(ha)	(%)	(ha)	(%)	(ha)	(%)	(ha)	(%)
Varieties	480	100	469	100	396	100	228	100
Chemical fertilizers	480	100	469	100	364	92	180	79
Vetiver grass hedgerows	139	29	94	20	218	55	89	39
Green manures	72	15	0	0	0	0	114	50
Intercropping	0	0	0	0	0	0	0	0

[1)] Estimated by farmers in each site during Participatory Monitoring and Evaluation (PM&E) in Aug 2002.

Table 16 shows how in Vietnam the number of households in the pilot sites adopting the various technology components increased over time, with most farmers adopting new varieties. This is partially due to the testing in FPR variety trials, but is also due to the planting of new varieties by non-participating farmers in or near the pilot sites. For instance, during 2002 and 2003, farmers in Van Yen district of Yen Bai province in North Vietnam planted a total of 500 km of double hedgerows of *Tephrosia candida* or *Paspalum atratum* to control erosion, and they planted about 3000 ha of new cassava varieties with improved fertilizer practices. This increased average yields from 10 t/ha to about 30 t/ha. **Figure 7** shows how the number of farmers in the pilot sites adopting various soil conservation measures increased year after year, initially mostly in Thailand but subsequently also in Vietnam.

Data in **Table 17** indicate that adoption of soil conservation practices in all sites in Vietnam increased yields, ranging from 13.5% in 2000 to 23.7% in 2002. As a result of the adoption of soil conservation practices, gross income, both per ha and per household, also increased very markedly over time. Results from both FPR trials and on-station research also indicate that the beneficial effect of contour hedgerows in terms of increasing yields and decreasing erosion increased over time (**Figure 5**) (Howeler *et al*., 2005). This is mainly because the planting of contour hedgerows, almost independent of the species used, will result in natural terrace formation, which over time reduces the slope and enhances water infiltration, thus reducing runoff and erosion. Well established hedgerows also become increasingly more effective in trapping eroded soil and fertilizers. Unfortunately, most FPR erosion control trials are conducted for only 1-2 years at the same site, so farmers do not quite appreciate the increases in beneficial effects that result over time. This, coupled with the fact that planting and maintaining hedgerows requires additional labor (and sometimes money for seed or planting material), while hedgerows take some land out of production and have initially little beneficial effect on yield, has hampered the more widespread acceptance and adoption of these soil conservation practices.

Table 15. Change in the use of new cassava production technologies[1)] in four pilot sites[2)] in Thailand from 1993 to 2002[2)] as a result of the Nippon Foundation Cassava project.

Technology component	Baan Khlong Ruam			Thaa Chiwit Mai			Sapphongphoot			Huay Suea Ten		
	1993	1995	2002	1995	1997	2002	1995	1997	2002	1995	1997	2002
Varieties	R90 (60%) R3 (30% R60 (10%)	R90 (60%) R5 (20%) KU50 (20%)	R5 (67%) R90 (19%) KU50 (12%) R72 (2%)	R1 (94%) R60 (3%) R5 (3%)	KU50 (41%) R60 (32%) R5 (22%) R90 (5%)	KU50 (81%) R5 (18%) R72 (1%)	R1 R60 R90	KU50 R5 R90	KU50 (91%) R90 (5%) R72 (3%) R5 (1%)	R1 R90 KU50	KU50 R5 R90	KU50 (54%) R5 (20%) R90 (15%) R72 (11%)
Chemical fertilizers	not apply	15-15-15 13-13-21	15-15-15 (35%) 13-13-21 (17%) 21-4-21 (13%) 14-4-24 (10%) 16-20-0 (5%) other (20%)	not apply	15-15-15	15-15-15 (50%) 13-13-21 (38%) other (12%)	not apply or 15-15-15 (little)	15-15-15 46-0-0	15-15-15 (44%) 46-0-0 (27%) 13-13-21 (4%) other (25%)	not apply or 15-15-15 (little)	15-15-15 and 16-8-8 mixed at 2:1 ratio	15-15-15 (47%) 16-8-8 (33%) 21-0-0 (12%) 46-0-0 (7%) 13-13-21 (1%)
Vetiver grass	not plant	46%	29%	not plant	3%	20%	not plant	70%	55%	not plant	32%	39%
Green manures	not plant	not plant	*Canavalia* (little) cowpea (little	not plant	not plant	*Canavalia* (little)	not plant	not plant	*Canavalia* (little) *Crotalaria* (little)	not plant	*Canavalia* (20%)	*Canavalia* (50%)

[1)] Date collected from Participatory Monitoring and Evaluation (PM&E) with farmers in Aug 2002; percentages are in terms of cassava area.

[2)] Baan Khlong Ruam village, Wang Soombuun district, Sra Kaew province; Thaa Chiwit Mai village, Sanaam Chaikhet district, Chachoengsao province Sapphongphoot village, Soeng Saang district, Nakhon Ratchasima; Huay Suea Ten village, Sahatsakhan district, Kalasin province

[3)] Nippon Foundation project started in these pilot sites around 1997, except in Baan Khlong Ruam where it started in 1995.

Table 16. Trend of adoption of new cassava technologies in the Nippon Foundation cassava project sites in Vietnam from 2000 to 2003.

	Number of households adopting			
Technology component	2000	2001	2002	2003
1. New varieties	88	447	1,637	14,820
2. Improved fertilization	64	123	157	1,710
3. Soil conservation practices	62	200	222	831
4. Intercropping	127	360	689	4,250
5. Pig feeding with cassava root silage	-	759	967	1,172

[1)]Number of project sites: 1999 = 9; 2000=15; 2001=22; 2002=25; 2003=34
Source: *Tran Ngoc Ngoan, 2008.*

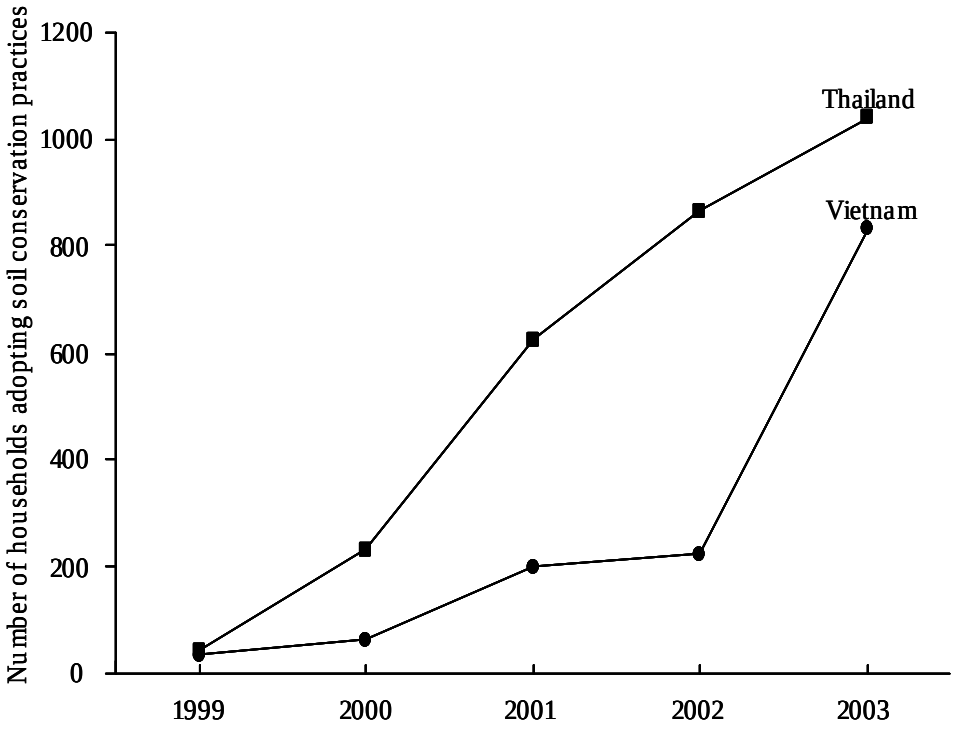

Figure 7. Number of farmers adopting soil conservation measures in their cassava fields in FPR pilot sites in Thailand and Vietnam from 1999 to 2003.

Table 17. Extent of adoption of soil conservation practices and the estimated increase in yield and gross income of farmers in the FPR pilot sites in Vietnam from 2000 to 2003.

Year	Number of farmers	Area with soil conser. (ha)	Cassava yield (t/ha)		Percent yield increase	Increase in gross income (mil VND)[2)]		
			Farmers' practice[1)]	With soil conservation		per ha	total	per household
2000	62	21.12	12.11	13.75	13.5	0.574	12.123	0.196
2001	200	59.87	16.50	19.95	20.9	1.112	66.596	0.333
2002	222	88.85	20.60	25.48	23.7	1.952	173.728	0.782
2003	831	612.00	20.60[3)]	25.48[3)]	23.7	1.561	955.699	1.150
Total	**831**	**612.00**					**1,208.146**	

[1)] Farmers' practice includes most new technologies except soil conservation

[2)] Fresh root price: in 2000 350 VND/kg
in 2001 350 VND/kg in north, 200 in central and 290 in south
in 2002 400 VND/kg
in 2003 320 VND/kg (estimated)

[3)] Yields estimated from 2002

***Source**: Tran Ngoc Ngoan, 2008.*

Table 18 shows in more detail how the adoption of various technologies increased over time in one commune in Pho Yen district of Thai Nguyen province where the project first started working in 1994. Since 1995 farmers have conducted FPR trials on new varieties, more balanced fertilization, intercropping, and erosion control. After some years of testing farmers initially adopted new varieties and intercropping in small areas of their land. This was followed by better fertilization and erosion control; the latter was adopted by only a small number of farmers as most cassava fields in the commune are on gentle slopes or on terraced land. It is clear that the adoption of new technologies increased yields significantly, of both the local variety Vinh Phu and the new varieties, mainly KM 95-3 and KM 98-7. The gradual increases in yield, from 8.5 t/ha in 1994 (see **Table 1**) to 36.8 t/ha in 2003 was accompanied by an increase in area planted using new technologies, resulting in about a 20-fold increase in net income and marked improvements in the livelihood of farmers in this commune.

Table 19 summarizes the extent of adoption of new cassava technologies in FPR pilot sites in 15 provinces of Vietnam in 2003 and the resulting increase in gross income due to higher yields obtained. Although balanced fertilization produced the greatest yield increase, it was not adopted over a very wide area. New varieties were most widely adopted resulting in the greatest increase in gross income. The total annual increase in gross income due to adoption of new technologies in the FPR sites was estimated at 1.67 million US dollars or $72.92 per household.

Table 18. Impact of the adoption of new cassava varieties and improved production practices on the livelihoods of farmers in Tien Phong commune, Pho Yen district of Thai Nguyen, Vietnam.

Year	Variety or practice[1)]	No. of farmers	Cassava area (ha)	Cassava yield (t/ha)	Peanut yield (t/ha)	Gross income[2)]	Production costs	Net income	Total net income
						——(mil. dong/ha)——			(mil.dong)
1994[3)]	Vinh Phu	115	50	8.5	-	3.40	2.93	0.47	23.50
	New varieties	0	-	-	-	-	-	-	-
			50						**23.50**
2000	Vinh Phu	NA[4)]	NA	21.5	-	NA	NA	NA	NA
	New varieties	25	1.31	30.9	-	15.45	4.36	11.10	14.54
	Intercropping	37	2.59	29.3	0.81	18.70	6.16	12.54	32.48
	Erosion control	4	0.20	24.7	-	12.35	4.66	7.69	1.54
			>4.10						**>48.56**
2001	Vinh Phu	61	2.17	22.7	-	11.35	4.36	6.99	15.17
	New varieties	122	4.70	29.0	-	14.50	4.36	10.14	47.66
	Intercropping	40	3.38	26.2	0.77	16.94	6.16	10.78	36.44
	Erosion control	4	0.20	NA	-	NA	NA	NA	NA
			10.45						**>99.27**
2002	Vinh Phu	18	0.64	25.4	-	12.70	4.33	8.37	5.36
	New varieties	100	5.16	33.7	-	16.85	4.33	12.52	64.60
	Intercropping	118	3.69	32.3	1.73	24.80	6.13	18.67	68.89
	Balanced fert.	48	2.95	33.4	-	16.70	4.83	11.87	35.02
	Erosion control	5	0.18	25.4	-	12.70	4.63	8.07	1.45
			12.62						**175.32**
2003	Vinh Phu	NA	NA	NA	-	NA	NA	NA	NA
	New varieties	225	17.00	36.8	-	18.40	4.33	14.07	239.19
	Intercropping	120	11.00	36.0	0.67	21.35	6.13	15.22	167.42
	Balanced fert.	54	3.40	33.6	-	16.80	4.83	11.97	40.70
	Erosion control	5	0.60	27.0	-	13.5	4.63	8.87	5.32
			>32.00						**>452.63**

[1)] In Tien Phong farmers traditionally grow mainly Vinh Phu variety but have now largely changed to KM 95-3 and KM 98-7; the new practices include intercropping with peanut, balanced fertilization of 10 t/ha of pig manure plus 80 kg N-40 P_2O_5-80 K_2O/ha, and erosion control by contour hedgerows of *Tephosia candida*

[2)] Price of cassava in 1994: 400 VND/kg fresh roots
Price of cassava in 2000-2003: 500 VND/kg fresh roots
Price of peanut in 2000-3003: 5,000 VND/kg dry pods

[3)] Data from RRA at the start of project

[4)] NA = data not available

Table 19. Extent of adoption of new cassava production technologies in FPR pilot sites in 15 provinces of Vietnam in 2003/04, the effect on cassava yields, and the increase in gross income resulting from the yield increase in those sites.

Technology component	No. of households	Area (ha)	Cassava yield (t/ha)		Increase in gross income ('000 US$)[2)]
			Farmers' practice[1)]	Improved technology	
1. New varieties	14,820	7,849	19.93	28.95	1,462
2. Balanced fertilization	1,710	607	21.37	30.50	114
3. Soil conservation practices	831	612	20.60	25.48	62
4. Intercropping	4,250	160	29.95	28.94	15 [4)]
5. Root and leaf silage for pig feeding	1,172	-[3)]	-	-	12
Total	**22,833**	**9,228**			**1,665**

[1)] Farmers' practice usually includes most new technologies except the technology being tested
[2)] based on a price of 320 VND/kg fresh roots in 2003/04; 1 US$ = 15,500 VND
[3)] 3,370 pigs
[4)] increase in gross income from the harvest of intercrops
Source: *Tran Ngoc Ngoan, 2008.*

ASSESSMENT OF IMPACT

In order to determine more precisely the effect of this project on adoption of new technologies, an impact assessment was made by an outside consultant. He organized focus group discussions and collected data from farmers in eight representative project sites - four sites in Thailand and four in Vietnam - as well as from farmers living within 10 km of those sites, who had not participated in the project. **Table 20** shows the percent of households (out of 767) who had adopted various technologies. New varieties were adopted[4] by nearly all cassava farmers in the eight sites in Thailand and by 70% of farmers in Vietnam; the use of chemical fertilizers had been adopted by 85-90% of households in the eight sites in each country; intercropping by nearly 60% of households in Vietnam, but by only 13% in Thailand. Contour ridging was adopted by about 30% of households in both Vietnam and Thailand, while contour hedgerows were adopted by 23% of households in Thailand and 25% in Vietnam; in Thailand these hedgerows were almost exclusively vetiver grass, while in Vietnam most farmers preferred the planting of *Tephrosia candida* or *Paspalum atratum* (Howeler, 2008), as these are easier to plant (from seed) and can also serve as a green manure and animal feed, respectively. Thus, it is clear that adoption of specific practices varies from site to site, depending on local conditions and traditional practices. **Table 20** also indicates that there were highly significant differences in the adoption of almost all the technologies between participating and non-participating farmers (with the exception of contour ridging and the use of chemical fertilizers in Vietnam), with participating farmers having a greater extent of adoption than non-participating farmers. In this case, "participants" were defined as farmers who had conducted at least one FPR trial and/or had participated in an FPR training course, while "non-participants" had done neither, but may have attended a farmer field day organized by the project. It can be seen

[4] Planted in 50% or more of the farmer's total cassava area

that new varieties and the use of chemical fertilizers were readily adopted by both participants and non-participants, while, adoption of soil conservation practices and intercropping was both less widespread and largely limited to participating farmers. This clearly points to the difficulty of achieving spontaneous and widespread adoption of soil conservation practices.

Table 20. Extent of adoption (per cent of households)[1)] of new technologies by participating and non-participating farmers (n=767) in the cassava project in Thailand and Vietnam in 2003.

	Thailand			Vietnam			Full sample		
Technologies	Partic. n=109	Non-partic. n=308	Total n=417	Partic. n=126	Non-partic. n=224	Total n=350	Partic. n=235	Non-partic. n=532	Total n=767
New Varieties									
- 100% in improved varieties	100	88.0	91.1	50.0	38.8	42.9	73.2	67.3	69.1
- 75% in improved varieties	0	11.7	8.6	5.6	6.7	6.3	3.0	9.6	7.6
- 50% in improved varieties	0	0.3	0.2	26.2	18.3	21.1	14.0	7.9	9.8
- 25% in improved varieties	0	0	0	4.0	5.4	4.9	2.1	2.3	2.2
- No improved varieties	0	0	0	14.3	30.8	24.9	7.7	13.0	11.3
	100	100	100***[2)]	100	100	100***[2)]	100	100	100***[2)]
Soil conservation practices									
- contour ridging	52	22	30***	35	31	33	43	26	31***
- hedgerows	60	10	23***	50	12	25***	54	11	24***
- no soil conservation	21	72	59***	23	58	45***	22	67	53***
Intercropping	28	8	13***	79	49	59***	55	25	34***
Fertilization									
- chemical fertilizers	98	86	89***	85	86	86	91	86	87**
- farm yard or green manure	55	25	33***	74	60	65**	65	40	48***
- no fertilizer	0	13	9***	12	8	9	6	11	9*

[1)] Percentages may total more than 100 percent as households can adopt more than one type of technology simultaneously

Significant differences between participants and non-participants. * P<=0.10; ** P<=0.05; *** P<=0.01

[2)] Level of significance refers to differences between participants and non-participants in terms of the categorical distribution, not to the level of adoption

Source: *Dalton et al., 2005.*

But how does adoption of these new technologies translate into higher yields and income? **Figure 8** shows the cassava yields that farmers reported before and after the project, corresponding more or less to the second phase of the project, or from 1999 to 2003. In Thailand the yields of participating farmers increased from 19.4 to 25.8 t/ha (33%), while yields of non-participating farmers increased from 15.5 to 20.3 t/ha (31%); in Vietnam project participants increased yield from 13.7 to 28.2 t/ha (106%), while non-participants increased their yields from 14.3 to 23.9 t/ha (67%) (Dalton *et al*., 2007). Thus, in both countries yields increased very markedly, but these increases were greater for participants than for non-participants, especially in Vietnam. For comparison, **Figure 8** also shows the increase in yield for the whole country, as reported by FAO, during approximately the same time period. Yields for the whole of Vietnam are considerably below those reported by the farmers in the focus groups; but the yield increases are similar to those reported by the non-participants. In Thailand the initial yields in the country were similar to those of non-participating farmers, but after-project yields were much higher for participants as well as nearby non-participants than for the country as a whole. This indicates that participating farmers benefited most from their experiences but that nearby farmers also benefited indirectly from the project.

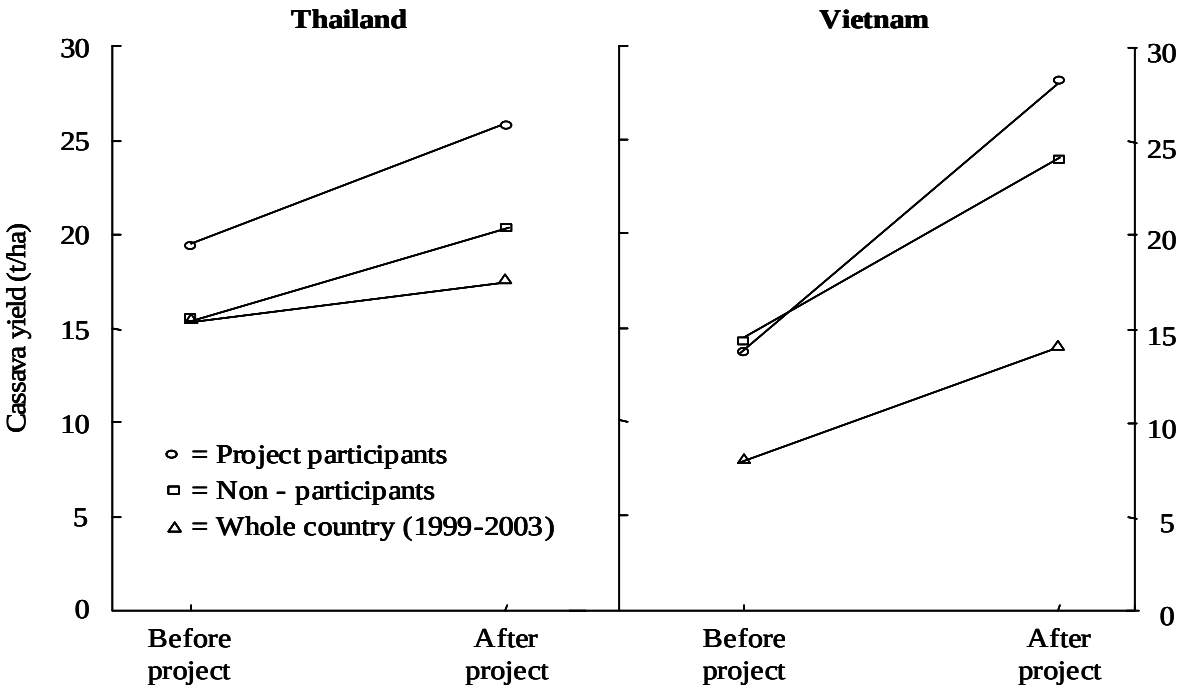

Figure 8. Average cassava yields of farmers participating in the Nippon Foundation cassava project or of near-by but non-participating farmers, before the project started and at the end of the project. Data are from PRRA census forms collected from 417 households in Thailand and 350 households in Vietnam. For comparison, the national average cassava yields in 1999 (before) and 2003 (after) are also shown.

Table 21 shows that during the ten years of the project the average cassava yields in all three countries increased; this increase ranged from 0.30 t/ha in China to 6.54 t/ha in Vietnam. The increased yields resulted in annual increases in gross income received by farmers of about 251 million US dollars in the three countries, and about 397 million US dollars in all of Asia. In addition, farmers in Thailand received higher prices due to the higher starch content of the new varieties. This was achieved not only by this project, but by the collaborative effort of many researchers, extensionists, factory owners and farmers, with strong support from national governments.

Table 21. Estimated increase in gross income of cassava farmers in China, Thailand, Vietnam and in all of Asia as a result of increased cassava yields in 2004 as compared to 1994.

	Total cassava area (ha)[1)]	Cassava yield (t/ha)[1)]		Yield increase (t/ha)	Cassava price ($/ton)	Increased gross income due to higher yields (mil. US$)
		1994	2004			
China	245,767	15.23	15.53	0.30	30	2.21
Thailand	1,057,338	13.81	20.28	6.47	26	177.86
Vietnam	388,600	8.44	14.98	6.54	28	71.16
Asia total	3,494,567	12.93	16.99	4.06	28	397.26

[1)] Data from FAOSTAT for 2004

[2)] In addition, farmers also benefited from higher prices due to higher starch content

***Source**: Howeler, 2010.*

RATE OF RETURN ON THE RESEARCH INVESTMENT

To calculate the internal rate of return (IRR) on investment of this project, we need to calculate the total costs and the total benefits that can be attributed directly to the project. The total costs of the project in Thailand and Vietnam were calculated as 2/3 of the Nippon Foundation project annual budget over a 10-year period, plus contributions for salaries of national staff and other expenses provided by the two national governments. These costs totaled about 3.5 million US dollars (Lila/Johnson *et al*., 2005).

Benefits were calculated by adding up the incremental yield increases obtained as a result of participation in the project (9.1 t/ha), by the adoption of contour hedgerows (2.7 t/ha) or of new varieties (up to 6.3 t/ha depending on the extent of adoption) multiplied by the average area in each village affected by either participation or the particular technology adopted. According to these calculations each village on average increased their cassava production by 1,895 tons as a result of the project. Since there were 67 project villages in Thailand and Vietnam and the price of fresh cassava roots was about 25 US dollars per ton, this translates into a total annual benefit of 3.2 million US dollars. If we assume a linear rate of adoption between 1998 and 2004 the project had an IRR of 33% over that period, or an IRR of 37% if we assume that adoption will continue at a similar rate until 2008 (Lilja/Johnson *et al*., 2005).

CONCLUSIONS

Research on sustainable land use conducted in the past has mainly concentrated on finding solutions to the bio-physical constraints, and many solutions have been proposed for improving the long-term sustainability of the system. Still, few of these solutions have

actually been adopted by farmers, mainly because they ignored the human dimension of sustainability. For new technologies to be truly sustainable they must not only maintain the productivity of the land and water resources, but they must also be economically viable and acceptable to farmers and the community. To achieve those latter objectives farmers must be directly involved in the development, adaptation and dissemination of these technologies. A farmer participatory approach to technology development was found to be very effective in developing locally appropriate and economically viable technologies, which in turn enhances their acceptance and adoption by farmers.

The conducting of FPR trials is initially time consuming and costly, but once more and more people are trained and become enthusiastic about the use of this approach – including participating farmers – both the methodology and the selected improved varieties or cultural practices will spread rapidly. The selection and adoption of those farming practices that are most suitable for the local environment and in tune with local traditions will improve the long-term sustainability of the cropping system, to the benefit of both farmers and society at large.

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CHAPTER 22

CASSAVA AGRONOMY: LAND PREPARATION, TIME AND METHOD OF PLANTING AND HARVEST, PLANT SPACING AND WEED CONTROL [1]

Tin Maung Aye[2]

INTRODUCTION

This chapter presents the highlights of cassava agronomy research, particularly on land preparation, planting times and methods, and plant spacing, in cassava based-cropping systems in tropical and sub-tropical Asia. It includes detailed data-based presentations and discussion of farmers' existing cassava based-cropping systems and testing of improved land preparation, different planting methods, time of planting and harvest, and plant spacing in small-holder farming systems. Site-specific on-farm research findings in target areas is discussed, with specific examples from upland areas in Asia.

Cassava-based Cropping Systems in Asia

Growing cassava is the most important livelihood or source of income for about eight million, mostly poor, farmers in Asia, covering about 4 million hectares. Approximately 35% of cassava in the world is currently being produced in Asia (FAOSTAT, 2011). In traditional upland farming systems, farmers often grow cassava together with other crops. For decades this has been a way of food security for these small-holder farmers. The specific cropping systems for cassava vary markedly from one part of Asia to another (Onwueme, 2002). Cassava can be planted either as a sole crop in a monoculture system or intercropped with other crops. Only recently some improvements have been introduced to farmers in some areas of Asia.

Land Preparation

In general, land preparation involves plowing, harrowing, and leveling the ground to make it suitable for crop establishment. Where cassava is traditionally grown as the first crop after clearing the land, no land preparation is required other than removal of bushes, shrubs, vines, etc. When the first rains have softened the ground, farmers loosen the soil in individual planting holes with a hoe or sharp spade, and plant cassava cuttings. For continuously grown cassava, as soon as one cassava crop has been taken out, the land can be tilled and preparations can be started for the next cropping season.

Since soil physical and chemical conditions influence the growth of cassava plants and their root yields, proper tillage is required for sustainable cassava production. Therefore, appropriate land preparation is one of the most important agronomic practices for successful cultivation. Various different methods of land preparation had a highly significant effect on the fresh root yield of cassava but not on the root starch content (Jongruyasuk *et al*., 2007). However, timely land preparation is also needed and the best time of tillage is required to achieve its maximum benefits. Soil should be cultivated when moist, but not too wet or too dry. Cultivation of very dry or very wet soil can break up the

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[1] For color photos see pages 779-782.

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soil structure, leading to poor drainage and aeration, surface crusting, and greater susceptibility to erosion. The land should be adequately prepared prior to planting of the cassava stakes.

Good land preparation involves the removal or incorporation of crop residues and any weeds or other vegetation that may compete with the cassava crop, either manually or through mechanical tillage. Tillage aims to turn over and loosen the topsoil and the compacted soil below, in order to achieve a good tilth for forming the mounds or ridges, and provide a uniform medium where storage root growth is not obstructed. After initial land preparation, soil samples can be collected for analysis, especially in the case of commercial large-scale cassava plantations. The soil analysis will determine the need for any soil amendments that must be incorporated before planting. The following land preparation should aim to incorporate any soil amendments, manure or chemical fertilizers that need to be applied before planting; and, depending on the location, may also incorporate residues remaining from the previous crop, which contribute to the build up of soil organic matter and provides nutrients for the following crop.

In the rainfed uplands, farmers widely use draft animals, such as water buffalo and oxen, as the power source for land preparation, while human labor is mostly used in the remote steep upland areas. On smaller farms, land is generally prepared by hoeing or by plowing with cattle or water buffalo (**Photos 1** and **2**). In Indonesia, land is often prepared by plowing with cattle followed by hand-ridging with hoe. In Kerala, India, small plots are generally prepared by hoe, making individual mounds for each plant. On steep slopes in Laos and southern China, land is cleared of vegetation by machete, followed by burning; land preparation is limited to making individual holes for planting each stake horizontally. However, power tillers and tractors have been introduced in many areas, particularly where improved industrial cassava varieties are planted to increase yields (**Photo 3**). Good land preparation can be achieved by thorough plowing and harrowing done a number of times, depending on soil conditions. It should incorporate all crop residues and weeds and create a soil structure that allows the cassava stakes to emerge rapidly and uniformly, and provide the young plants ready access to the vital resources of nutrients, water and oxygen.

Experiments conducted in two locations in Colombia, one on sloping land in Mondomito, Cauca Department, and one on flat land in Carimagua in the Eastern Plains, indicate that in both locations planting cassava without land preparation resulted in the lowest yields, followed by the treatment of alternating 1 m prepared with 1 m unprepared contour strips. In Mondomito highest yields were obtained with the use of a tractor mounted rototiller, while in Carimagua using a disk harrow followed by a disk plow, disk harrow and ridger (Howeler *et al*., 1993).

In a field with 25% slope at CATAS in Hainan island of China, hand preparation of only planting holes resulted in similar yields as twice plowing and disking, but markedly reduced soil erosion. Zero tillage followed by direct planting in small holes reduced yields and slightly increased erosion (Zhang Weite *et al*., 1998). In Thailand no-tillage (zero tillage) and using herbicides to control weeds sometimes resulted in high yields if weed growth was not aggressive (Jongruyasuk *et al*., 2007.). However, in very weedy plots or in compacted soil, zero tillage generally resulted in lower yields and difficulty in planting, weeding and harvesting. Therefore, no-tillage systems generally produced low cassava yields but may have improved the soil's physical conditions as compared to conventional tillage (**Tables 1** and **2**). Most cassava farmers in Thailand now prepare their land by contract plowing with a 3-disk plow followed by a 7-disk harrow, which in turn may be

followed by a ridger. An experiment to determine the most effective method of mechanical land preparation, conducted for three or four consecutive years in three locations in Thailand, showed that the best method of land preparation differed among the three locations, but that overall the use of a subsoiler followed by a chisel plow, or the standard practice of using a 3-disk plow followed by a 7-disk harrow and ridger, produced the highest yields (**Table 3**). In Khaw Hin Sorn plowing with a 3-disk plow and 7-disk harrow, either alone or preceded by a subsoiler produced the highest yields, which were significantly higher than those obtained using zero tillage or using only a subsoiler. In Rayong subsoiling followed by a 3-disk plow, and in TTDI subsoiling followed by a chisel produced the highest yields (Watananonta *et al*, 2006; R.H. Howeler, unpublished).

Table 1. The effect of various methods of land preparation on the average fresh root yield and root starch content of Rayong 90, planted for three years at Rayong FCRC in Thailand from 1992/93 to 1994/95.

Land preparation treatments	Fresh root yield (t/ha)	Starch content (%)
No-tillage	13.63 d	26
Two times with 7-disk plow	17.86 b	25
One time with 7-disc plow followed by animal ridging	16.86 bc	26
Two times plowing with 3-disk plow, followed by 7-disk plow	20.43 a	26
Two times of animal ridging	15.22 cd	26
One time of subsoiler followed by 7-disk plow	15.54 cd	25
Cassava harvester followed by 7-disk plow		
F-test	**	NS
cv.(%)	14.32	6.74

***Source**: Jongruyasuk et al., 2007.*

Table 2. The effect of various methods of land preparation on the fresh root yield and starch content of Rayong 5 at Rayong FCRC in 1995/96.

Land preparation treatments.	Fresh root yield (t/ha)	Starch content (%)
No-tillage	10.66 c	21.67
Two times with 7-disk plow	19.28 a	21.22
One time with 7-disk plow followed by animal ridging	14.46 bc	22.25
Two times plowing with 3-disk plow, followed by 7-disk plow	16.31 ab	24.27
Two times of animal ridging	16.06 ab	22.80
One time of subsoiler followed by 7-disk plow	13.63 bc	20.29
Cassava harvester followed by 7-disk plow	15.96 ab	22.15
F-test	*	NS
cv. (%)	19.75	9.16

***Source:** Jongruyasuk et al., 2007.*

Table 3. Summary of land preparation trials conducted for three or four years at three locations in Thailand from 2001 to 2006.

Treatments	Fresh root yield (t/ha) [1)]													Average 3 locations
	Rayong				TTDI				Khaw Hin Sorn					
	1st year	2nd year	3rd year	**Av.**	1st year	2nd year	3rd year	**Av.**	1st year	2nd year	3rd year	4th year	**Av.**	
1. No tillage; Glyphosate	11.46	23.94	22.39	**19.26**	19.91	26.07	15.14	**20.37**	32.71	24.90	14.87	16.75	**22.31**	**20.65**
2. Chisel plow; Glyphosate	12.03	24.92	22.84	**19.93**	17.78	25.10	10.93	**17.94**	34.18	21.80	15.27	21.26	**23.13**	**20.33**
3. Subsoiler; Glyphosate	13.70	24.21	22.62	**20.18**	16.31	24.32	10.10	**16.91**	33.01	24.48	16.04	12.24	**21.44**	**19.51**
4. Subsoiler + chisel; Glyphosate	14.85	25.99	25.04	**21.96**	21.87	28.71	14.20	**21.59**	37.65	23.12	20.23	23.00	**26.00**	**23.18**
5. Cassava harvester; Glyphosate	14.60	25.82	23.43	**21.28**	16.08	25.52	12.52	**18.04**	39.50	26.66	23.58	25.64	**28.84**	**22.72**
6. 3 disk plow	13.66	22.76	23.82	**20.08**	18.00	-	-	-	-	-	-	-	-	-
7. Subsoiler + 3 disk plow	17.57	28.54	27.68	**24.60**	16.59	-	-	-	-	-	-	-	-	-
8. 3 disk plow + 7disk harrow	11.93	23.00	24.02	**19.65**	18.15	23.31	8.92	**16.79**	41.99	27.67	25.95	27.38	**30.75**	**22.40**
9. 3 disk plow+7disk harrow + contour ridging	17.47	24.60	25.35	**22.47**	18.32	26.57	8.53	**17.81**	46.35	25.40	23.55	18.84	**28.53**	**22.94**
10. 3 diskplow+7 disk harrow + up-down ridging	19.50	25.86	23.41	**22.92**	17.52	-	-	-	-	-	-	-	-	-
11. Subsoiler + 3 disk plow; Glyphosate	-	-	-	-	-	-	-	-	36.24	26.42	23.94	25.98	**28.14**	-
12. Subsoiler + 7 disk harrow; Glyphosate	-	-	-	-	-	25.35	11.91	-	-	-	-	-	-	-
13. Subsoiler + 7disk harrow	-	-	-	-	-	24.90	10.04	-	28.65	28.39	22.11	14.11	**23.31**	-
14. Subsoiler+3 disk plow+7disk harrow	-	-	-	-	-	26.40	10.88	-	38.95	29.16	27.43	24.82	**30.09**	-
Average	**14.68**	**24.96**	**24.06**	**21.23**	**18.05**	**25.63**	**11.32**	**18.49**	**36.92**	**25.80**	**21.30**	**21.00**	**26.25**	

[1)] Average yield of four varieties planted in subplots*; **Source**: R.H. Howeler, unpublished.*

The use of heavy machinery, such as tractors, for land preparation may compact the subsoil, producing a hard pan or compaction layer. Spoor (2000) indicated that continuous cultivation at constant depth creates a zone of high compaction (known as hard pan or plow sole) in the sub-surface soil. The depth of this zone will depend on the farmer's practices. This problem is greater on clay soils than on sandy soils. A hard pan can slow down drainage, causing water logging, poor development of beneficial organisms, and poor root growth, which subsequently leads to poor cassava plant growth and poor quality storage roots. Plowing breaks the soil crust, while deep plowing or subsoiling breaks the hard pans, improving water penetration and aeration. The regular use of a subsoiler will help to break the plow sole and improve internal drainage, which tends to improve plant growth during the height of the rainy season and increase yields (Watananonta *et al.*, 2006). Excessive compaction, on the other hand, resulted in a high soil bulk density, high penetrometer resistance, a low water infiltration rate and low hydraulic conductivity, as well as markedly reduced root yields (Silpamaneephan, 1994).

Loosening up the soil increases the oxygen content, which favors the development of microorganisms that decompose organic matter. Good land preparation also helps control weeds, pests and diseases. Plowing is the most effective method for weed control. Control of weeds is necessary because they compete strongly with crops for moisture, nutrients and light. Before planting, plowing cuts off perennial weed shoots and exposes many roots to sunlight so they dry out and die. Moreover the plow leaves the surface rough and porous, increasing the amount of water that enters the soil and helps to control erosion. However, overworking a field with a disk plow can be disastrous as it leaves the soil surface too fine, and loose. Overworked soil easily loses moisture and the lower half of the plowed soil layer may end up as hard as before it was plowed because it gets compacted by the heavy machinery.

There are three main land preparation methods, namely flat method, ridges and furrows, and mounding (**Photos 4, 5, 6** and **7**). Depending on the topography, sloping land can be prepared either on the flat, or with mounds, ridges or furrows. Lowlands (in valleys) should be prepared as mounds or ridges above the normal ground level to reduce the effect of water-logging. On deep, well-drained soils, cassava planting may be done on the flat, on ridges or mounds or in furrows. The shallow furrows are usually made by oxen or buffalos, after which the cassava stakes are thrown in the furrows at a constant distance from each other and covered by soil for a horizontal planting position (**Photo 5**). For vertical or inclined planting, cassava stakes can be inserted directly into the prepared land, either on the flat, on ridges or mounds.

For the ridge and furrow method, the land is usually plowed and harrowed by animals or tractor (usually at a depth of 20-25 cm) and then ridges or furrows are made and cassava stakes are subsequently planted on ridges or in the shallow furrows. On sloping land ridges should be made along the contours, to maximize rain infiltration and minimize erosion. On flat land, ridges may be oriented East-West for maximum light interception, or in any direction for convenience of furrow irrigation wherever this method of irrigation is possible. Ridges are typically about 30-45 cm high, but may be higher in low-lying areas to maximize drainage. They are usually between 0.90 to 1.2 m apart.

Mounds are often preferred by farmers working totally with hand tools, such as hoe and spade. The mound method gathers the soil into heaps and is commonly used in

traditional cassava cropping systems in Asia. Mounds range from 25 to 75 cm high with broader bases, and the space between mounds varies from 0.5 m to 2 m. In some areas, broad raised beds are also used.

Different methods of land preparation did not show any significant difference in root yield of cassava. In light textured-soils the flat method of land preparation, in heavy textured-soils mound method of land preparation, and under irrigation conditions ridge and furrow is suggested in India (Ravindran, 2006). Generally cassava planting on ridges is better in the rainy season, but planting on the flat is better in the dry season (Howeler, 1987).

Land preparation depends on climate and soil type, topography, cropping systems, and labor availability. No one set of guiding standards is appropriate for all situations. However, minimum tillage is thought to be a more appropriate technique for light-textured sandy soils. Tillage must be done in a way that will assure adequate protection of soil and water resources. Ultimately, minimizing land preparation costs and increasing yields through better land preparation are necessary to achieve sustainable cassava production.

Time of Planting and Harvest

In tropical and sub-tropical Asia, planting of cassava can be done throughout the year if there is enough available soil moisture. Therefore, the best time of planting would be after the on-set of rains (pre-monsoon) under rainfed conditions, but the crop can be planted year-round under irrigated conditions. Many researchers found that cassava yields are seriously reduced if either low rainfall or low temperatures are limiting growth during the period of 3-5 months after planting. Howeler (2001) indicated that the best time to plant cassava not only depends on the climatic conditions at time of planting but also on climatic as well as marketing conditions at time of the expected harvest. In those areas where the root price depends on the starch content, farmers want to try to maximize both yield and starch content at time of harvest. However, prices also depend on market conditions and are usually highest in the off-season, i.e. when most farmers do not harvest. Thus, some farmers may want to sacrifice some yield in order to benefit from higher prices in the off-season.

a. Tropical regions

In tropical regions with distinct dry and wet seasons and a mono-modal rainfall distribution, the best time to plant is early in the wet season, i.e. as soon as enough soil moisture allows for adequate germination of planted stakes. **Figure 1** shows that in Rayong, Thailand, highest yields were obtained with planting in May, at the start of the rainy season. In those areas with a bimodal rainfall distribution, such as in Kerala, India, planting at the start of the second rainy season, i.e. in August or September, will also result in high yields (George *et al*., 2001). In some parts of Asia, such as in Myanmar, farmers plant cassava at two different times, in the beginning of the monsoon season (i.e. May to June) and during the post-monsoon season (i.e. October to November) (Aye and Oo, 2010). In the southern hemisphere the wet and dry seasons are reversed in comparison with the northern hemisphere, and the wet season generally starts in November-December and ends in April-May. In that case, highest cassava yields are obtained when planted in December (Wargiono *et al*., 2001).

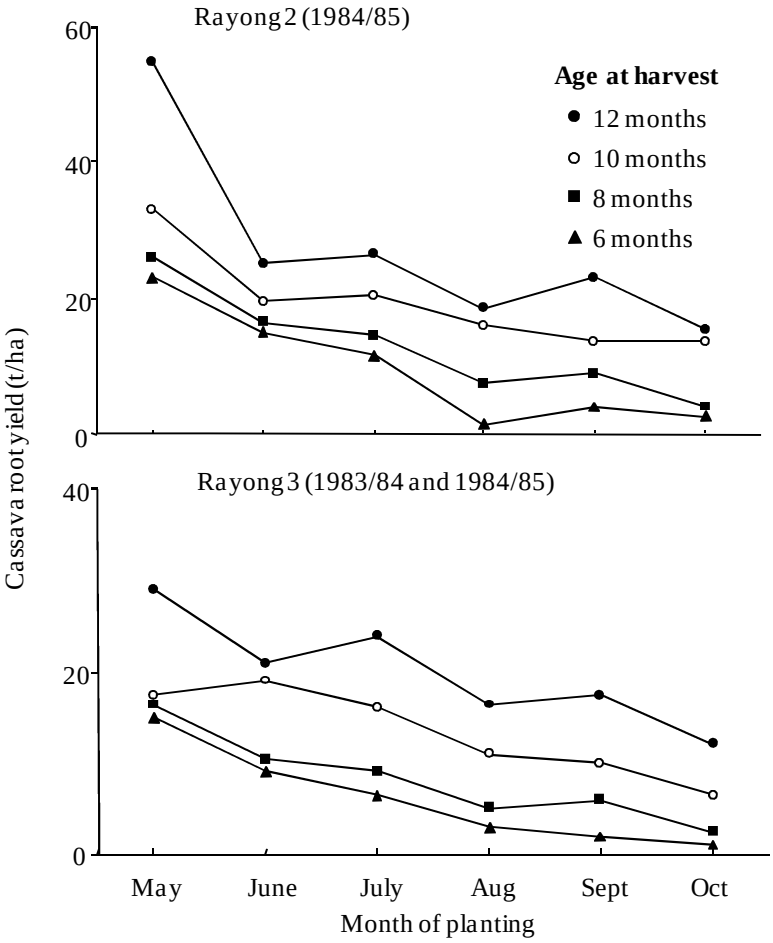

Figure 1. Effect of month of planting and age at harvest on root yields of cassava cultivars Rayong 2 and Rayong 3, planted at Rayong Field Crops Research Center, Thailand in 1983-1985.
***Source**: Tongglum et al., 2001*

However, high yields may also be obtained when cassava is planted towards the end of the wet season. **Table 3** shows that highest yields in Rayong, Thailand were obtained when cassava was planted in Aug-Nov. In this case, plants get well established during the last months of the rainy season, grow slower during the dry season and have an additional period of fast growth during the following wet season. In this case, weed competition tends to be less severe as plant canopies are already well-established during the early part of the second wet season

Figure 1 and **Table 4** indicate that root yields generally increase with increasing plant age at harvest, at least up to 18 months. Root starch content also tends to increase with plant age up to 9-10 month but may decrease sharply at the early part of the wet season as plants relocate starch from the roots to plant tops during resprouting.

Table 3. Fresh root yield (t/ha) of recommended cassava cultivars when planted at different periods at Rayong Field Crops Research Center, Thailand, 1987-1988.

	Cultivars				Average
Planting periods	Rayong 1	Rayong 3	Rayong 60	Rayong 90	
April-May	18.56	19.94	23.31	24.00	21.44 c[1)]
June-July	20.81	24.25	27.63	29.31	25.50 ab
August-Sept	22.31	24.44	32.31	27.81	26.75 a
Oct-Nov	21.81	26.62	30.19	26.06	26.19 a
Dec-Jan	19.38	20.38	29.44	23.87	23.25 bc
Feb-March	20.75	20.50	26.25	25.44	23.25 bc
Average	**20.62 d**	**22.69 c**	**28.19 a**	**26.06 b**	

[1)] Mean separation: DMRT, 0.01

Source: *Tongglum et al., 2001*.

Table 4. Average fresh root yield of Rayong 1 as affected by age at harvest when planted at Rayong Field Crops Research Center, Thailand in 1975-1979.

Age at harvest (months)	Fresh root yield (t/ha)	Dry root yield (t/ha)	Starch yield (t/ha)	Starch content (%)
8	16.19 f[1)]	6.44 f	2.31 f	14.3
10	23.06 e	8.31 e	4.81 e	20.9
12	31.31 d	10.69 d	5.94 d	19.0
14	37.56 c	13.06 c	7.38 c	19.6
16	41.50 b	15.00 b	8.69 b	20.9
18	45.25 a	16.44 a	9.19 a	20.3

[1)] Mean separation within each column: DMRT 0.01

Source: *Tongglum et al., 2001.*

Table 5 and **Figure 2** indicate that total rainfall during the 4th to 11th month of the crop cycle was best correlated with root and starch yield when the crop was harvested at 11 months after planting (MAP), but starch content was best correlated with total rainfall during the 6th to 9th month, and was negatively correlated with rainfall during the 10th and 11th months.

Table 5. Correlation coefficients between cassava root yield, starch content and starch yield, as well as dry soil losses due to erosion and rainfall during certain periods in the cropping cycle when cassava, cv Rayong 90, was planted at bimonthly intervals for three consecutive cropping cycles on 4.2% slope in Rayong Research Center in Thailand from 1994 to 1998.

Parameters	Correlation Coef. (r)	%P
Cassava root yield vs rainfall from the 4th-11th MAP[1)]	0.7025	0.001
Cassava root yield vs rainfall from the 3rd-11th MAP	0.6726	0.002
Cassava root yield vs rainfall from the 2nd-11th MAP	0.6005	0.008
Cassava root yield vs rainfall from the 1st-11th MAP	0.5115	0.030
Cassava root yield vs rainfall during the 1st MAP	-0.4258	0.078
Cassava root yield vs rainfall from the 1st-2nd MAP	-0.4146	0.087
Root starch content vs rainfall from the 6th-9th MAP	0.8298	0.000
Root starch content vs rainfall from the 5th-9th MAP	0.7981	0.000
Root starch content vs rainfall from the 6th-8th MAP	0.7966	0.000
Root starch content vs rainfall from the 10th-11th MAP	-0.1290	NS
Root starch content vs rainfall during the 11th MAP	-0.0772	NS
Starch yield vs rainfall from the 4th-11th MAP	0.7411	0.000
Starch yield vs rainfall from the 4th-10th MAP	0.7096	0.001
Starch yield vs rainfall from the 5th-11th MAP	0.7090	0.001
Starch yield vs rainfall from the 5th-10th MAP	0.6950	0.001
Dry soil loss (erosion) vs rainfall from 1st -3rd MAP	0.6016	0.008
Dry soil loss (erosion) vs rainfall from 1st -4th MAP	0.5515	0.018
Dry soil loss (erosion) vs rainfall from 1st -5th MAP	0.5290	0.024
Dry soil loss (erosion) vs rainfall from 1st-2nd MAP	0.5087	0.031

Note: cassava was harvested after 11 months

[1)] MAP = month after planting;

***Source**: Howeler, 2001*.

b. Subtropical regions

Cassava is also grown in subtropical regions, such as southern China and North Vietnam. These regions are characterized by cold and dry winters (with occasional frost at higher latitudes) and hot and wet summers with relatively long daylight. **Figure 3** shows that cassava yields were little affected by date of planting when cassava was harvested at 12 months, but that yields markedly declined when planted in late summer (Aug-Nov) and harvested after 8 months in April to July. When harvested at 8 MAP, both root yields and starch content were lowest when roots were harvested during the hot months of June-July. In that case, root yields were positively and highly significantly correlated with both temperature and rainfall during the 3rd to 5th month after planting, i.e. at time of maximum growth rate of cassava (**Figure 4**), while starch content was negatively correlated with temperature and rainfall during the last month before harvest (**Figure 5**).

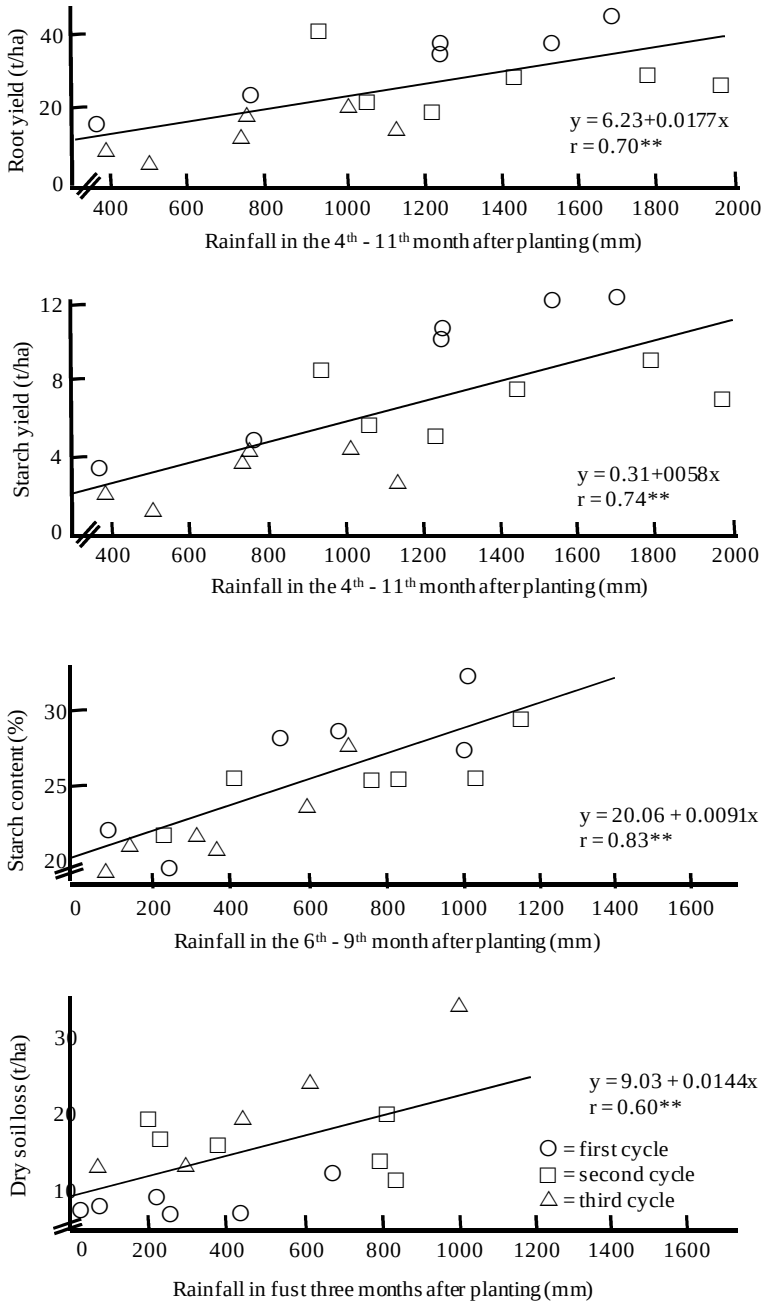

Figure 2. Linear regressions between cassava root yield, starch yield, starch content and dry soil loss due to erosion and the rainfall received during certain periods of the crop cycle when cassava, cv. Rayong 90, was grown at bimonthly intervals for three complete cropping cycles on 4.2% slope at Rayong Research Center in Thailand from 1994 to1996.
***Source**: CIAT, 1998b.*

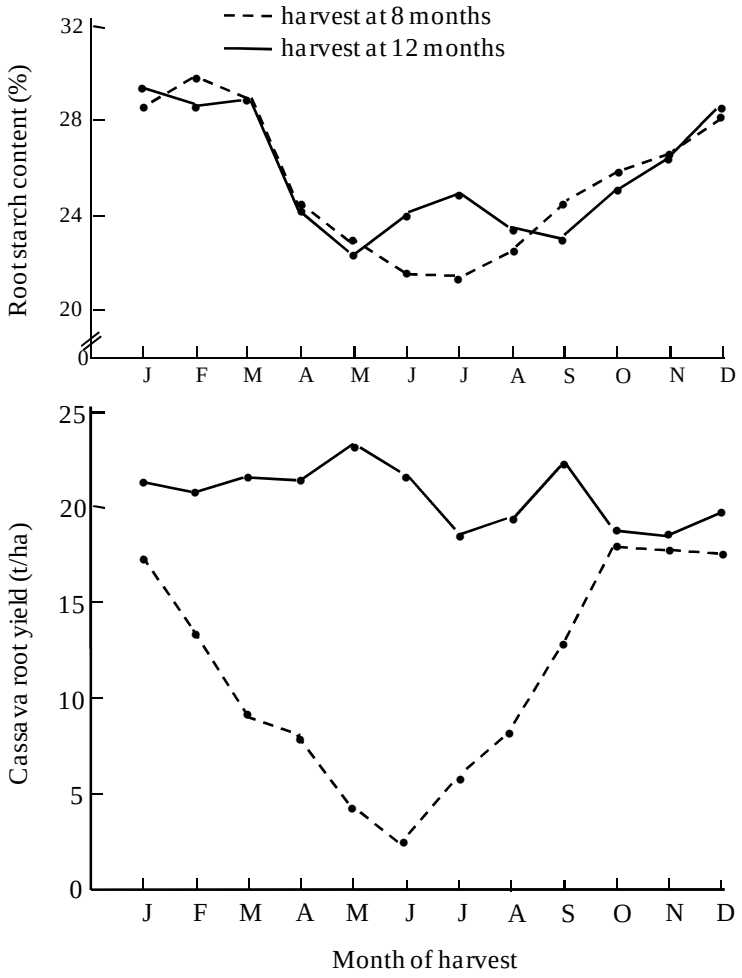

Figure 3. Cassava root starch content (top) and root yield (bottom) averaged over three varieties and three cropping cycles, when planted during different months of the year at CATAS, Danzhou, Hainan, China, and harvested after either 8 or 12 months.
Source: *Zhang Weite et al., 1998.*

It may be concluded that highest yields are generally obtained when cassava is planted as early as possible in the wet season or in early spring, while starch contents are highest when plants are harvested in the middle of the dry season. At planting time there should be enough soil moisture to get at least 80-90% germination, while soils should not be so wet as to prevent adequate aeration and root formation.

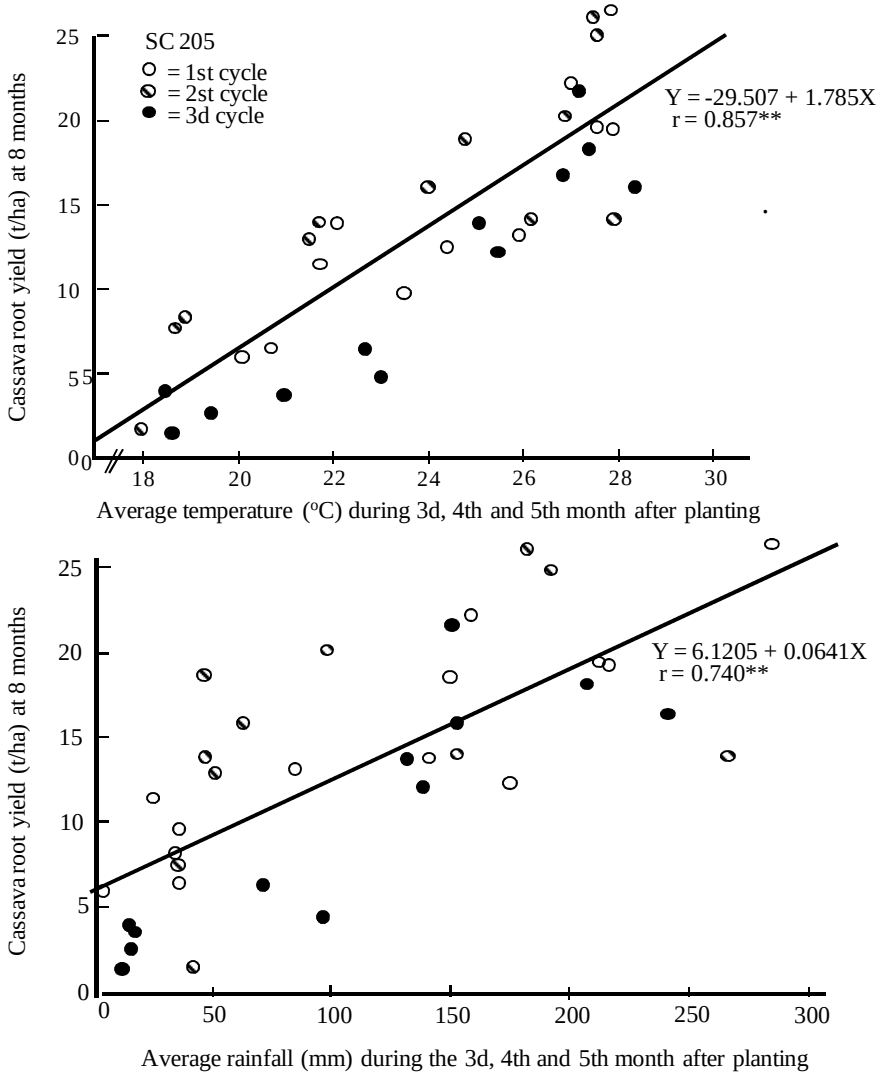

Figure 4. Linear regression between root yield of cassava, cultivar SC 205, harvested at 8 months, and the average mean temperature (top) or rainfall (bottom) during the 3d, 4th and 5th month after planting in CATAS, Danzhou, Hainan, China. Data are for 36 monthly plantings from 1990 to 1993.
***Source:** Zhang Weite et al., 1998.*

Planting times of cassava have to be regulated according to prevailing climatic conditions. Cassava should be planted just before the rains or after the rains start, or in the late raining season. Generally cassava should be planted as early as possible because early planted stakes sprout and establish well, and receive sufficient moisture during the growth period. In areas where very low temperatures are possible, the cuttings are planted as soon as danger of frost has past. Therefore, delayed planting may lead to reduced yield of cassava. The common times of cassava planting in different parts of Asia is shown in **Table 6.**

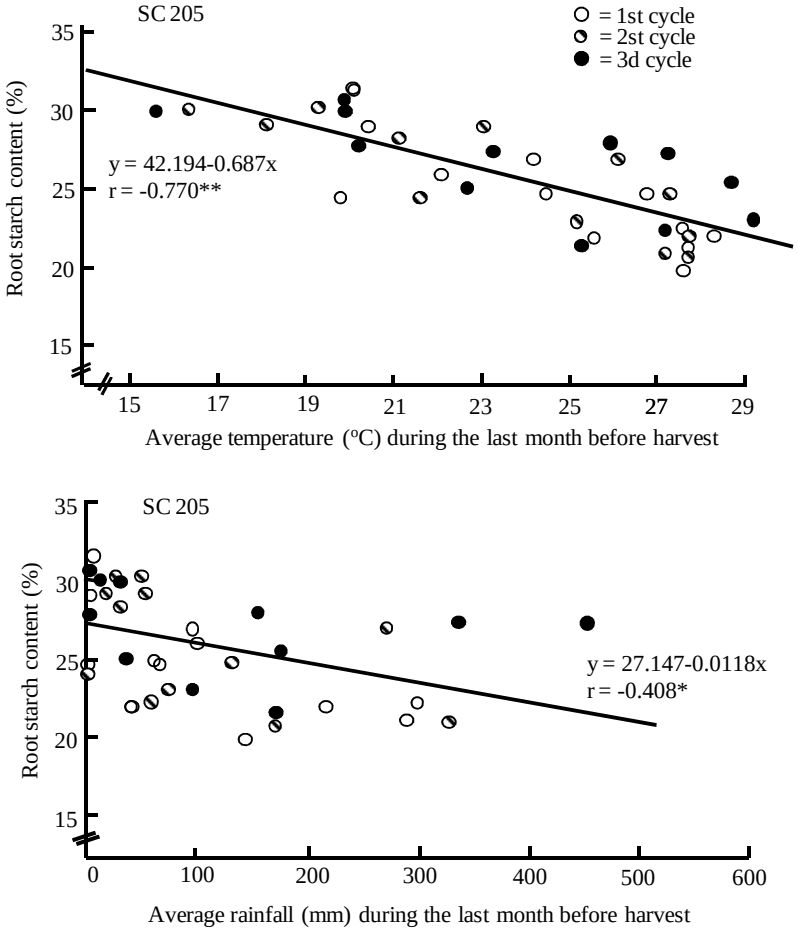

Figure 5. Linear regression between root starch content of cassava, cultivar SC 205, harvested at 8 months, and the average temperature (top) or rainfall (bottom) during the last month before harvest in CATAS, Danzhou, Hainan, China. Data are for 36 monthly plantings from 1990 to 1993.
Source: *Howeler, 2001.*

Planting Methods

In Asia three planting methods are used, i.e. horizontal, inclined (slanted) and vertical.

Horizontal method: The entire stake is placed horizontally and buried at a depth of 5 to 20 cm (usually about 10 cm) in the ground (**Photo 8**). This method produces shallower roots than slanted and vertical planting.

Inclined method: The stake is placed 2/3 of its length in the ground and at an angle ranging from about 45º to 60º (**Photo 9**).

Vertical method: The stake is pushed vertically and about 1/2 of its length into the ground (**Photo 10**). With this method the stake sprouts quicker than with the other two methods, but it produces deeper roots than the horizontal or inclined planting methods.

Table 6. Common times of cassava planting in different parts of Asia.

Country	Time of planting
Cambodia	Uplands (March-July) and Flood plain (November-December)
China	Beginning of rainy season (March-April)
East Timor	Early rainy season
India	Tamil Nadu: Rain-fed (October-November), irrigated (January-April);
	Kerala: early rainy season (March-April)
Indonesia	Early rainy season up to the start of the following dry season (December-May)
Laos	Before and during early rainy season (February-June)
Myanmar	Year-round, early rainy season (May-June) and late rainy season (Oct-Nov)
Philippine	Early rainy season (June) or late rainy season (November)
Thailand	Year-round, late dry season (February-April) and late rainy season (Oct-Dec)
Vietnam	Early rainy season (May-June) or late rainy season (October-November)

If the soil is loose and friable, stakes can be planted vertically or slanted by pushing the lower part of the stake about 5-10 cm into the soil. Stakes can also be planted horizontally at 5-7 cm depth by digging individual holes, or by making a long furrow, laying the stakes down and covering with soil (see **Photo 5**). Planting vertically or slanted generally produces higher yields than planting horizontally, especially during periods of drought (Howeler, 2001), and the vertical method is suitable in sandy soils and under erratic rainfall. In sandy clay loam soils in Rayong, Thailand, planting vertically or inclined produced significantly higher root yields than planting horizontally (**Table 7**); this was especially the case when stakes were planted in the early dry season (Nov), when horizontal planting resulted in a significantly lower rate of germination (Tongglum *et al.*, 2001).

Research conducted in two locations in China indicate that vertical planting resulted in the highest germination percentage, but that slanted planting produced the highest yields (**Table 8**). A similar result was obtained in Colombia where vertically planted stakes always germinated the best, especially when rainfall was limited during the first 30 days after planting; in that case the root yields were significantly higher than with horizontal or slanted planting (CIAT, 1979). With vertical planting callus formation around the cut surface developed more uniformly, which resulted in the uniform distribution of roots around the base of the cassava plant (Ravindran, 2006). Slightly different results were recently obtained in Cambodia (Sopheap *et al*., 2010) where vertical, slanted and horizontal planting methods with one stake per hill all produced similar yields. Planting one stake per hill significantly increased yields as compared to the traditional practice of planting two

stakes per hill, slanted in opposite directions (**Table 9**). Planting stakes horizontally is common in heavy clay soils or with zero- or minimum-tillage methods of land preparation. When the soil is well prepared and friable, planting vertically or slanted is faster than planting horizontally, but care should be taken that the eyes or buds on the stakes face upward; with horizontal planting this is of no concern.

Table 7. Effect of stake position, stake length, and planting depth on cassava yield, planted in both the rainy and dry season at Rayong Field Crops Research Center, Thailand. Data are the average of three years, 1987-1989.

	Rainy season (May-August)			Early dry season (November)		
Treatments	No. plants survived ('000/ha)	Root yield (t/ha)	Starch content (%)	No. plants survived ('000/ha)	Root yield (t/ha)	Starch content (%)
Method of planting						
-Ridge	14.57 a	14.98 a	16.64 a	10.69 b	14.69 a	18.63 a
-No ridge	14.43 a	13.47 a	16.66 a	12.09 a	14.96 a	18.65 a
F-test	NS[3)]	NS	NS	**	NS	NS
Stake position						
-Vertical	14.87 a	16.04 a	17.03 a	13.04 a	17.74 a	19.04 a
-Inclined	14.89 a	15.46 a	17.14 a	11.99 b	16.40 b	18.68 a
-Horizontal	13.74 b	11.08 b	15.85 b	9.31 c	10.32 c	18.17 b
F-test	**[1)]	**	**	**	**	**
Stake length (cm)						
-20	14.55 a	14.52 a	16.67 a	10.58 b	14.53 a	18.51 a
-25	14.41 a	13.54 b	16.69 a	13.02 a	15.41 a	18.87 a
F-test	NS	*[2)]	NS	**	NS	NS
Planting depth (cm)						
-5-10	14.43 a	13.90 a	16.61 a	9.74 b	13.14 b	18.21 b
-15	14.56 a	14.43 a	16.73 a	12.71 a	16.17 a	18.97 a
F-test	NS	NS	NS	**	**	**

No interaction between methods and treatments in all characters

[1)] and [2)]: Mean within a column separated by DMRT at 0.01 and 0.05 %, respectively

[3)] NS = not significantly different.

Source: *Tongglum et al., 1992.*

Table 8. Effect of stake planting position and ridging on cassava yield and germination at 1 month in GSCRI, Nanning, Guangxi, and in CATAS, Danzhou, Hainan, China. Data are the average for SC201 and SC205 in CSCRI, and for SC205 and SC124 at CATAS.

Planting Position		GSCRI (1990-1992) Germination[1] (%)	GSCRI (1990-1992) Root yield[2] (t/ha)	CATAS (1994) Root yield (t/ha)
Horizontal				
	-ridging	61.5	11.7	20.0
	-no ridging	67.4	10.9	18.6
Inclined				
	-ridging	66.4	13.0	25.3
	-no ridging	78.1	11.5	16.9
Vertical				
	-ridging	82.8	11.1	19.4
	-no ridging	85.8	11.2	18.5

[1)]Average of 1991 and 1992 (no data taken in 1990)
[2)]Average of 1990 and 1992 (no harvest in 1991 due to drought)
Source: *Zhang Weite et al., 1998.*

Table 9. Effect of planting methods on cassava root yields (t/ha) in 12 FPR trials conducted by farmers in four provinces of Cambodia in 2006/07 and 2007/08 using KM 94 variety.

Planting method	Year 2006/07					Year 2007/08							
	BB1	BB2	KC1	KC2	KS	BB1	BB2	KC1	KC2	KC3	KS	PV	**Aver.**
Vertical	30.0	34.2	25.0	35.0	19.5	13.3	55.0	21.3	20.0	33.3	6.7	19.6	**26.1**
Horizontal	36.7	28.8	30.0	37.5	35.9	10.8	46.0	22.5	19.6	36.7	4.6	23.8	**27.7**
Inclined +one stake	25.0	27.5	42.5	35.8	14.7	20.8	63.0	22.1	18.8	31.7	7.5	32.5	**28.5**
Inclined +two stakes	27.5	23.3	25.0	25.8	11.8	7.5	42.1	20.7	16.7	30.8	5.0	12.1	**20.7**

Note: BB = Battambang (4 sites); KC = Kampong Cham (5 sites); KS = Kampong Speu (2 sites); PV = Preah Vihear .

Source*: Sopheap et al., 2010.*

The planting depth should also be regulated according to prevailing environmental conditions. Shallow planting at low moisture results in poor establishment and low yield. In sandy soils with dry conditions, cassava stakes should be planted deep and in clay soils with moist conditions, cassava stakes should be planted shallow. Deep planting makes harvesting difficult, thus increasing harvesting costs of cassava roots

Plant Spacing

Farmers practice different spacings with the distance between plants in the row varying from 50 to 150 cm. A plant density of 10,000-15,000 plants per ha in general results in good yields. The optimum plant spacing varies from uplands to lowlands, and depends on whether cassava is a sole crop or is intercropped with other crops. In upland areas farmers traditionally grow cassava together with other crops such as maize, peanuts, banana, etc. The distance between cassava plants will differ depending on the type of intercrops, but generally ranges from 100 to 400 cm. In monocropping, the spacing of cassava is usually between 80 and 100 cm within and between rows. In Thailand, the plant spacing in monocropped cassava will vary from 1 x 1 m to 1 x 0.5 m (Howeler, 1988).

The plant spacing depends mainly on: variety, climatic conditions, soil fertility of specific locations and cultural practices. However, there is no universal recommendation for the plant spacing of cassava. Branching and vigorous cassava varieties will need wider spacing compared to less branching and less vigorous varieties. The branching habits of different cassava varieties are shown in **Figure 6**. Cassava grown on very fertile soils will need wider spacing compared to cassava grown on infertile soils.

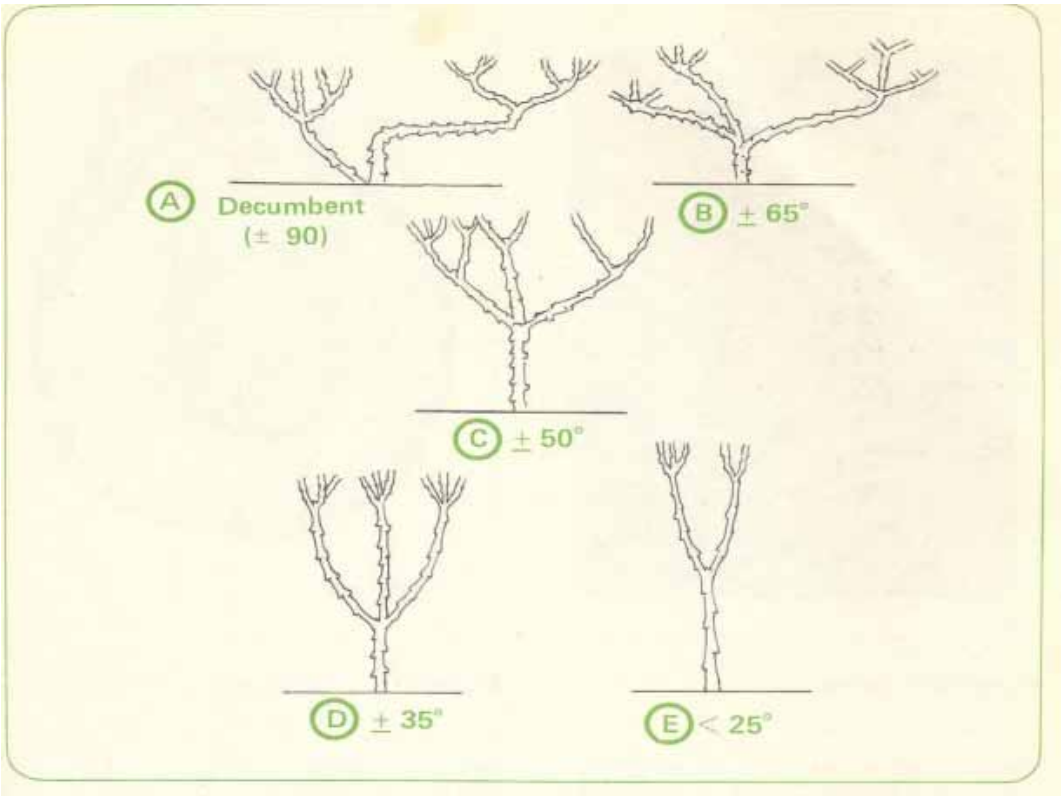

Figure 6. Branching habits of different cassava varieties.

Weed Control

Cassava yields can be markedly reduced by competition from weeds. It has been reported that yields may be reduced 25-50% if weeds are not controlled, particularly at the early growth stage (Tirawatsakul, 1983). However, the negative effect of weeds on cassava yields depends on the weed population in any particular location, on the cassava variety and plant population used, and on the type of weed control. **Figure 7** shows that highest yields in two varieties were obtained when cassava was kept weed-free during a 10-month period with herbicides, i.e. a pre-emergence application of diuron and alachlor, followed by a shielded post-emergence application of paraquat, and that optimal production was reached at 15,000 plants/ha.

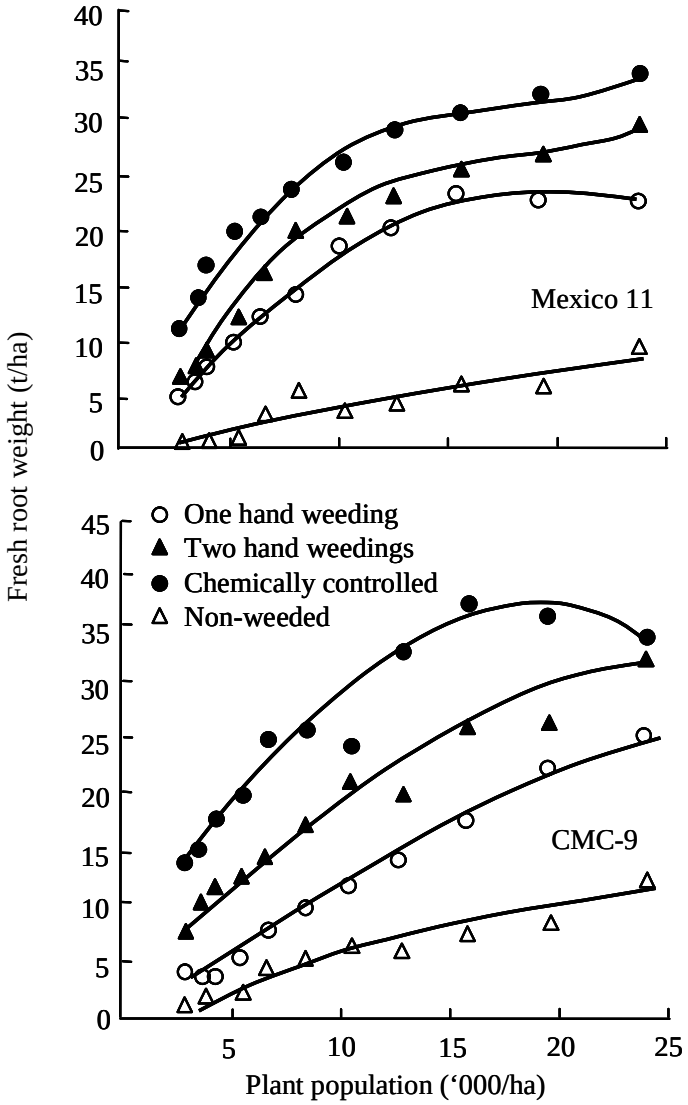

Figure 7. Effect of cassava plant population and weed control system on the fresh root yields of Mexico 11 and CMC 9 harvested at 10 months after planting at CIAT, Cali, Colombia. ***Source:*** *Doll and Piedrahita, 1978.*

When the traditional methods of one to two hand weedings were used, the highest yields were obtained at 15,000 to 20,000 plants/ha for Mexico 11 and between 15,000 and 25,000 plants/ha for the highly branched variety CMC 9. Higher crop densities will compensate for the effects of weed competition when the weed control system is not sufficiently intensive. By keeping the crop totally weed-free, especially during the early growth stages, fewer plants per hectare were needed to achieve maximum production. When weeds were not controlled at all, yields were extremely low; nevertheless, yields increased as plant density increased.

Tongglum *et al*. (1992) also studied the effect of frequency of weeding on the yields of two recommended varieties, Rayong 3 and Rayong 60. The results show that two times of hand weeding, at 1 and 2 months after planting, gave the best results for both varieties (**Table 10**). The results also indicate that weeding costs varied according to the planting season, the cost being much higher when cassava was planted in the early rainy season than in the dry season.

Table 10. Cassava fresh root yield and weeding costs as effected by the frequency of hand weeding when cassava cultivars Rayong 3 and Rayong 60 were planted at Rayong Field Crops Research Center in the beginning of the rainy and dry seasons of 1991.

Treatment	Rainy season		Dry season	
	Root yield (t/ha)	Weeding cost (US$/ha)	Root yield (t/ha)	Weeding cost (US$/ha)
Varieties				
-Rayong 3	21.44 b	111	22.88 b	57
-Rayong 60	28.00 a	94	30.81 a	53
F-test	*[1)]	-	*	-
Weeding times				
-No weeding	4.81 b	0	23.63	0
-1&2 months	26.69 a	77	24.88	9
-1, 2& 3 months	29.00 a	85	25.38	14
-1, 2, 3 &6 months	27.94 a	127	26.06	57
-1, 2, 3, 6 & 9 months	31.44 a	118	29.56	104
-As necessary	28.81 a	106	31.56	90
F-test	**[2)]	-	NS[3)]	-

[1)] and [2)] Mean within a column separated by DMRT at 0.05 and 0.01%, respectively.
[3)] NS = not significant
Source: *Tongglum et al., 1992.*

During 1993-1995, additional experiments on weed control for cassava were conducted at Khon Kaen Field Crops Research Center in the northeast of Thailand. Rayong 1, Rayong 60 and Rayong 90 cultivars were planted in both the early (May-June) and late (Sept-Oct) rainy seasons. Plots were weeded for 0, 2, 3 or 4 months as compared to a typical "farmer" practice of manual weeding only at 2 MAP and without fertilizer application.

Results shown in **Table 11** indicate that weed control is extremely important during the first two months after planting, but weed control beyond 2 MAP did not significantly increase yields any further. The highest yields were obtained when plots were maintained weed-free for 3 MAP. Thus, when cassava is planted in either the early or late rainy season, cassava needs to be free of weeds for about 2-3 months after planting to produce high yields.

Table 11. Effect of weed control on the yields (t/ha) of three cassava varieties planted in the early (ER) and late (LR) rainy seasons at Khon Kaen, Thailand, in 1993/94 and 1994/95.

	1993/94		1994/95		Average 2 years		
	ER	LR	ER	LR	ER	LR	Average 2 seasons
Cultivars (C)							
-Rayong 1	28.33	19.53	10.86	17.23	20.97	18.38	19.67
-Rayong 60	23.33	27.68	15.11	14.59	19.22	21.13	20.18
-Rayong 90	25.03	21.88	11.33	12.25	18.18	17.06	17.62
F-test (C)	NS	*	*	NS	NS	*	NS
Weed-free period (W)							
-0 month (check)	2.61	13.48	4.49	5.63	5.83	9.56	7.69
-2 months	31.98	26.43	16.71	15.52	24.34	20.98	22.66
-3 months	34.71	26.03	13.84	19.20	24.28	22.61	23.44
-4 months	31.47	24.96	13.73	17.54	22.59	21.25	21.93
-farmers' practice[1)]	27.07	24.25	13.39	15.54	20.23	19.89	20.06
LSD (0.05) for W	6.73	7.38	4.97	5.82	5.51	4.70	3.56
F-test (W)	**	**	**	**	**	**	**
F-test (CxW)	NS	NS	**	NS	NS	NS	NS

[1)] farmers' practice = manual weed control at 2 months with no fertilizer applied.
Source: *Khon Kaen Field Crops Research Center, Annual Report 1995.*

Traditionally weed control was done by animal and hand labor, which accounted for 40% of total labor used in cassava production in Thailand (Sinthuprama and Tiraporn, 1984). Due to the high cost and lack of labor, several experiments on chemical weed control were conducted during 1987-1991 with the objective of minimizing the number of times and cost of weed control in cassava. The results, shown in **Table 12**, indicate that the pre-emergence herbicide Metolachlor, applied at a rate of 1.56 kg ai/ha, could control 90% of the weeds during the first three months after planting, and this treatment resulted in a high yield at the lowest weeding cost. However, cassava yields, weeding costs and net income were similar when cassava was weeded twice with bullocks, followed by a post-emergence application with the contact herbicide Paraquat (commercial name Gramoxone).

Table 12. Effect of various chemical weed control methods in cassava (Rayong 1) on yield and economic benefits at Rayong Field Crops Research Center, Rayong, Thailand, in 1987/1988.

Treatment	Root yield (t/ha)	Gross income (US$/ha)	Weeding cost (US$/ha)	Net income[1)] (US$/ha)
1. Metolachlor (1.56 kg a.i./ha); PE[2)]	26.82 a[3)]	955	230	725
2. Oxyfluorfen (1.56 kg a.i./ha); PE	21.26 b	757	234	523
3. Metolachlor (1.56 kg a.i./ha); PE-B +Paraquat (0.50 kg a.i./ha); ST	25.76 ab	917	234	683
4. Metolachlor (1.56 kg a.i./ha); PE +once bullock cultivation +Fluazifop-buty1(0.38 kg a.i./ha); PE	25.66 ab	914	268	646
5. Metolachlor (1.56 kg a.i./ha); PE +Fluazifop-buty1(0.38 kg a.i./ha); ST	27.00 a	961	258	703
6. Twice bullock cultivation +Paraquat (0.50 kg a.i./ha); ST	26.84 a	956	237	719
F-test	**	-	-	-

[1)] Root price = US$ 35.6/ton
[2)] PE = Pre-emergence
PE-B = Pre-emergence, band spraying
ST = Spot treatment
Herbicide application rates are in kg active ingredient/ha.
[3)] Mean within a column separated by DMRT at 0.01% level.
Source: *Tirawatsakul et al., 1988.*

Different pre- and post-emergence herbicides have different degrees of selectivity for cassava and will kill different types of weeds. **Table 13** shows the names and recommended dosages used and their relative selectivity for cassava. In general, the lower dosage shown in the table is used when cassava is grown in light-textured soil, and the higher dosage is used in heavy-textured soils where leaching of the herbicide into the root zone of cassava is less likely. The pre-emergence herbicides shown in **Table 13** can be applied right after cassava planting (up to three days). Even in the vertical planting position, in which part of the planted stake is exposed, the herbicide can be sprayed overhead right over the stakes as long as the axillary buds have not yet sprouted. If the spraying is delayed and these buds have already sprouted, the herbicide should be band applied with a shielded nozzle to prevent the herbicide touching the sprouting plants. Since most post-emergence herbicides are not selective for cassava, they should be only band applied with a plastic or metal shield over the nozzle to prevent the spray from hitting the lower stem and leaves. Without this precaution, these herbicides can seriously affect the further growth of cassava (Leihner, 2002).

While Oxifluorfen is very effective in controlling weeds and is intermediately selective for cassava and peanut, it will seriously affect the growth of intercrops like maize, cowpea, mungbeans and common beans (*Phaseolus vulgaris*). When cassava is intercropped with any of the latter crops it is recommended to use a mixture of Oxadiazon

+ Metolachlor (0.5 + 1.0 kg a.i./ha) or Linuron + Metolachlor (0.25 + 1.0 kg a.i./ha) applied either before or after planting the intercrops. In order to reduce the possible damage to the intercrops it is recommended to use only 50% of the dosage normally used for monocropped cassava. This will slightly reduce their effectiveness in controlling the weeds, but once the intercrops are well established their foliage will greatly reduce further weed growth (Lopez and Leihner, 1980). Other combinations of herbicides used in various intercropping systems with cassava are shown in **Table 14.**

Table 13. Herbicides used for the control of weeds in cassava.

Technical name	Commercial name	Selectivity for cassava	Time of application [1)]	Dosage of CP/ha [2)]	Type of weeds controlled
Diuron	Karmex	intermediate	Pre	2.0-3.0 kg	broadleaved
Alachlor	Lazo	high	Pre	3.0-4.0 lit	grasses
Fluometuron	Cotoran	intermediate	Pre	4.0-5.0 lit	broadleaved
Oxifluorfen	Goal	intermediate	Pre	2.0-4.0 lit	broadleaved/grasses
Metribuzin	Sencor	intermediate	Pre	1.0-1.5 lit	grasses
Linuron	Afalon	intermediate	Pre	2.0-3.0 kg	broadleaved/grasses
Trifluralina	Treflan	high	Ibp	2.5-3.5 lit	broadleaved/grasses
Metolachlor	Dual	high	Pre	3.0-4.0 lit	grasses
	Karmex + Lazo	intermediate	Pre	1.0-1.5 + 1.5-2.0	broadleaved/grasses
	Cotoran + Lazo	intermediate	Pre	1.0-2.5 + 1.5-2.0	broadleaved/grasses
	Goal + Lazo	intermediate	Pre	1.0-2.0 + 1.5-2.0	broadleaved/grasses
	Afalon + Lazo	intermediate	Pre	1.0-1.5 + 1.5-2.0	broadleaved/grasses
	Karmex + Dual	intermediate	Pre	1.0-1.5 + 1.5-2.0	broadleaved/grasses
	Cotoran + Dual	intermediate	Pre	1.0-2.5 + 1.5-2.0	broadleaved/grasses
	Goal + Dual	intermediate	Pre	1.0-2.0 + 1.5-2.0	broadleaved/grasses
	Afalon + Dual	intermediate	Pre	1.0-1.5 + 1.5-2.0	broadleaved/grasses
Glyphosate	Roundup	not select.	Post	2.0-3.0 lit	broadleaved/grasses
Glufosinate	Basta	not select.	Post	1.0-3.0 lit	broadleaved/grasses
Fluazifop	Fusilade	high	Post	1.0-3.0 lit	grasses
Paraquat	Gramoxone	not select.	Post	2.0-3.0 lit.	broadleaved/grasses

[1)] Ibp = Incorporated before planting; Pre = pre-emergence; Post = post-emergence

[2)] CP = commercial product; lower dosage for use in light-texture soils and higher dosage in heavy-texture soils

***Source** : Calle, 2002.*

Besides the above mentioned manual (with hoe) and chemical control there are also several other ways to control, or at least reduce, weed competition in cassava. This includes the selection of a variety with vigorous early growth and good quality planting material, optimum plant density, and the use of well-balanced and band- or spot- applied fertilizers that will stimulate rapid early growth and canopy closure. In addition, intercropping cassava with fast growing crops like maize, cowpea, peanut, mungbean or various melons will also greatly reduce weed competition by shading out the weeds.

Finally, farmers often use tractor- or animal-drawn implements, like cultivators, to control weeds between the cassava rows, which are often followed by hoeing between plants within the row. This practice usually starts between 15 and 30 days after planting

and continues at about monthly intervals until the crop's canopy closure prevents the further use of this equipment.

Table 14. Pre-emergence herbicides used for crops grown in association with cassava.

Product or mixture	Dosage (kg a.i./ha) [1)]	Time of application	Selective for crops association with cassava
Linuron + fluorodifen	0.25-0.50 + 1.50-2.10	Post planting	Common bean, cowpea and mungbean
Linuron + metolachlor	0.25-0.50 + 1.00-1.50	Post planting	Common bean, cowpea, mungbean, peanut and maize
Oxadiazon + alachlor	0.25-0.50 + 0.90-1.40	1-2 weeks before or after planting	Maize
Diuron + alachlor	0.80-1.20 + 0.90-1.40	Post planting	Maize and taro
Oxifluorfen	0.25-0.50	1-2 weeks before or after planting	Peanut

[1)] The doses indicated are used as follows: low doses on light-textured soils and high doses on heavy textured soils. Quantities individually indicated for each product are combined to obtain the tank mix.

***Source**: Lopez and Leihner, 1980.*

Harvesting Methods

Traditionally, cassava is harvested by cutting off the top growth about 20 cm from the ground and then pulling on the remaining stump of the stem until the roots come out of the ground. In heavy or very dry soils this may require some digging around the roots with a spade, shovel, or hoe. Recently, farmers in Thailand have used some simple harvesting tools to grab the lower stem or stump. The metal tool is attached to a wooden or metal pole that can be used as a lever to more easily pull the roots out of the ground, normally without the need for any digging of the soil (**Photo 11**).

In larger plantations or in heavy soil in Thailand, cassava is now often harvested by a tractor-mounted harvesting tool that digs under the roots and lifts the root clumps out of the soil and onto the soil surface **(Photo 12)**. These root clumps are then gathered and the roots are cut off the remaining part of the stem and carried in a basket to a wagon or tractor-trailer for transport to a chipping and drying yard or to a starch factory. In other countries the roots may be carried in bamboo baskets on a shoulder pole to the house.

CONCLUSIONS

Cassava is a relatively easy crop to grow. It can grow and give reasonable yields in low fertility soils and in drought-prone areas with little risk of complete crop failure. However, to obtain better root yields and have sustainable production systems, the crop should be well-managed. The crop should be planted and harvested at an optimum time of the year. The important factors to consider when planting cassava are time of year, suitable land preparation and tillage methods, planting methods, optimum plant spacing and adequate weed control during the first 2-3 months after planting.

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CHAPTER 23

CASSAVA AGRONOMY: INTERCROPPING SYSTEMS [1]

Tin Maung Aye[2] and Reinhardt Howeler[3]

INTRODUCTION

Intercropping cassava with short-duration crops is a common practice among smallholder farmers in many tropical countries. These intercrops are useful because they supply either food or additional income, especially at times when the cassava crop can not yet be harvested; they may fix N and supply other nutrients to the topsoil; they may protect the soil from the direct impact of rainfall, and may reduce the speed of runoff water when the cassava canopy is not yet closed, thus reducing soil erosion; and they may reduce weed growth during the early stages of cassava development. However, intercrops need to be carefully managed in order to reduce the competition with cassava, for light, water and nutrients. This is usually done through modifications of the plant spacing or planting pattern of both crops, by adjusting the relative time of planting, and by fertilizing each crop adequately to maximize yields.

Types of Intercropping Systems

Growing two or more crops at the same time in the same field is usually described as an "intercropping system". However, these can still be subdivided into four different subsystems:

1. ***Mixed Intercropping***, in which usually several crops are grown mixed and randomly distributed in the same space, and these crops may be planted and harvested at different times according to their specific characteristics.
2. ***Row Intercropping***, in which two or more crops are grown simultaneously in a regular arrangement with a well-defined planting pattern, consisting usually of one or more rows of a short-duration crop in parallel rows between rows of the long-duration crop.
3. ***Relay Intercropping,*** in which one or more crops are planted within an existing crop in such a way that the final stage of the first crop coincides with the initial development of the other crops.
4. ***Strip Cropping,*** in which two or more crops are grown simultaneously in the same field, but in separate and alternating strips that are wide enough to allow independent cultivation but narrow enough to obtain some crop interaction.

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[1] For color photos see pages 783-785.

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Advantages and Disadvantages of Intercropping

Intercropping is usually practiced by small-holder farmers who have only small areas of land from which to feed or sustain a family. In this case, land and capital are the major constraints while labor may be rather abundant. These farmers have to maximize the total productivity of the land by optimizing the growth factors such as light, water and nutrients. Growing two or more crops together has the following advantages:

- The different crops provide a greater food variability such as carbohydrates from grain and root or tuber crops; protein from grain legumes; and vitamins and fiber from vegetables
- Increased yield stability or income and reduced risk of total crop failure
- Reduced incidence of pests and diseases
- Reduced weed competition
- Reduced soil loss by erosion by providing an early ground cover between the rows of the slow-growing long-duration crop
- More efficient use of land and labor, the latter being needed for different operations throughout the year
- Increased yield and total net income per unit area of land

However, intercropping also has certain disadvantages:

- It reduces the possibility of using mechanization for planting, weeding and harvesting, as well as the use of certain herbicides to control weeds and the application of fertilizers
- It may complicate the management of each crop individually
- It requires more labor per unit area
- Intercrop competition is likely to reduce the yield of each individual crop, although this is generally compensated for by an increase of the total value of all crops included in the system

Intercropping systems must be designed to maximize the total net income of the system, to increase the various advantages and decrease the disadvantages mentioned above. This will require the careful selection of the various crops to be planted, the most suitable varieties of each crop, the most effective plant densities and planting arrangements, the relative time of planting each crop, the most effective fertilization, amounts and balance of nutrients and times of application, as well as their distribution among the various crops.

Selection of Suitable Crops for Intercropping with Cassava

The selection is highly site-specific, depending on the soil and climatic conditions, as well as on local tastes and traditions. Farmers tend to select crops on the basis of differences in growth habits and growth duration. Having a slow initial growth, cassava can best be intercropped with crops having a rapid growth and early to medium growth duration, such as cowpea (*Vigna unguiculata*), peanut (*Arachis hypogaea*), mungbean (*Vigna radiata*) and maize. Crops with different rooting patterns and growth cycle improve the use of water stored in different soil layers. The various crops should also have different times of maximum water and nutrient usage and different nutritional requirements. Thus,

cassava tends to need mainly K for root formation while cereal crops require mainly N and grain legumes mainly P and K.

Intercropping cassava with short-duration grain legumes has the advantage of providing both carbohydrates from the cassava roots and protein from the grain legumes. The latter may also fix N, and cassava may benefit from this symbiosis. The selection of early maturing grain legumes, such as mungbean, peanut, bush-type common beans (*Phaseolus vulgaris)* and cowpea (*Vigna unguiculata*), has the advantage that the grain legumes are harvested before the cassava closes its canopy and neither crop suffers too much from interspecific competition. In this case, the association of a long-duration crop (cassava) with a short-duration crop results in a higher total yield due to better utilization of both space and time. Also, at the end of its growth cycle when many lower leaves have dropped off and new leaf production has slowed down, enough light may again be available between cassava rows to plant a second intercrop, depending also on the availability of soil moisture at this stage of development (Leihner, 1983) (**Figure 1**).

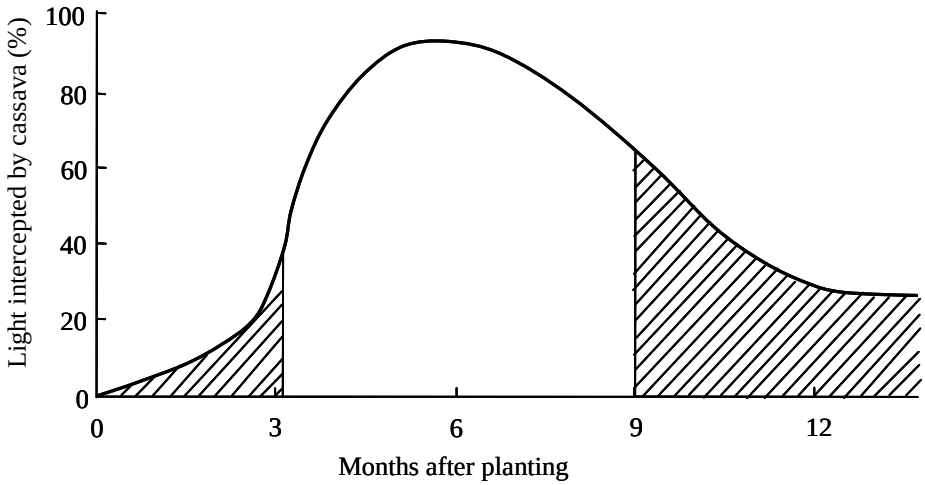

Figure 1. Interception of light by cassava during its vegetative cycle and possible periods for intercropping.
***Source**: Leihner, 1983.*

During the early stage of cassava development the selected intercrops should have either an erect or prostrate growth habit, but not be of the climbing type, and the crop should have a growth duration of <100 days to prevent excessive competition with cassava. At the later stage of cassava development, climbing types such as climbing beans (*Phaseolus vulgaris*), lima bean (*Phaseolus lunatus*) or velvet bean (*Stizolobium deeringianum* or *Mucuna pruriens*) can be selected, as these can climb on the cassava stems without causing serious competition. If it is desirable to harvest both crops at the same time, the climbing intercrops should have a growth duration of <120 days.

Cassava is also often interplanted between the rows of recently planted tree crops, such as rubber, coconut and cashew nut. As the trees grow and produce more and more shade, the number of cassava rows growing between the rows of trees is generally reduced until the shading of trees does not justify the further planting of intercrops. When cassava is planted under mature coconut trees, the yield of cassava tends to be greatly reduced, mainly due to excessive shading.

Commonly Used Intercropping Systems in Asia

Intercropping systems vary markedly from country to country as well as among different regions within the same country, depending on the soil and climatic conditions, especially the length of the rainy and dry seasons. The most commonly used systems are shown in **Table 1.**

Table 1. Intercropping systems with cassava in Asia.

Country	Associated Crops
Cambodia	Upland rice, maize, cashew nut, rubber
China	Maize, watermelon, sweet potato, peanut, rubber
East Timor	Maize, peanut, vegetables, banana
India	Maize, cowpea, vegetables, coconut
Indonesia	Upland rice, maize, soybean , cowpea, mungbean, peanut, coconut, rubber
Lao PDR	Upland rice, maize, Job's tear, peanut
Myanmar	Maize, peanut, common bean, banana
Philippines	Maize, peanut, sweet potato
Thailand	Maize, rubber, coconut, cashew nut
Vietnam	Maize, upland rice, peanut, black bean, rubber, cashew nut, coffee, tea

Probably the most intensive intercropping systems are found in the wetter zones of West Java and Sumatra of Indonesia. Here cassava is intercropped with simultaneously planted upland rice between cassava rows and maize between plants in the cassava row. Once the upland rice and maize are harvested at about four months after planting, a short-duration grain legume, such as mungbean, soybean (*Glycine max*), cowpea or peanut, are planted in the interrow space previously occupied by rice. If rainfall permits, a fourth intercrop, such as mungbean or peanut is planted in the space previously occupied by the harvested grain legume. In East Java, on the other hand, the dry season is longer and cassava can not be intercropped by more than one crop, usually maize.

In South Vietnam cassava is often intercropped with maize or planted among young rubber or cashew trees, while in North Vietnam the crop is often intercropped with peanut or black bean (cowpea).

In Guangxi province of China, cassava is often intercropped with maize, peanut, sweet potato or watermelon, while in Hainan province the crop is often interplanted among young rubber trees or bananas.

In Thailand cassava is only occasionally intercropped with maize or grain legumes due to lack of labor, but the crop is sometimes planted for a few years among young rubber or coconut trees.

Improvements in Cassava Intercropping Systems

Several factors should be considered in the selection of crops and management practices to maximize the outputs of intercropping systems.

Plant type and/or growth habit

Cassava varieties may differ in their growth habits, some having vigorous early growth and early branching, while others are more erect with medium- to late-branching. This may also vary with fertility of the soil; in soils low in K plants tend to be short and

highly branched , showing a prostrate growth habit, while plants growing in soils high in N are tall an show vigorous early growth. To minimize the shading of low-growing grain legumes by cassava, the latter should have an erect and late branching growth habit, but to avoid the shading of cassava by fast-growing intercropped maize, the former should have a vigorous early growth with medium- to late-branching.

Relative time of planting

The intercrops can be planted at the same time as cassava, or one or more weeks before or after planting cassava, depending on the vigor of each crop, as well as on the relative income expected from each crop. When the income from the intercrop is expected to be high, these crops may be favored by planting before the planting of cassava, and *vice versa.* However, in general, the greatest total yields are obtained when both crops are planted at the same time, or with a difference in planting date of only 1-2 weeks (**Figure 2**).

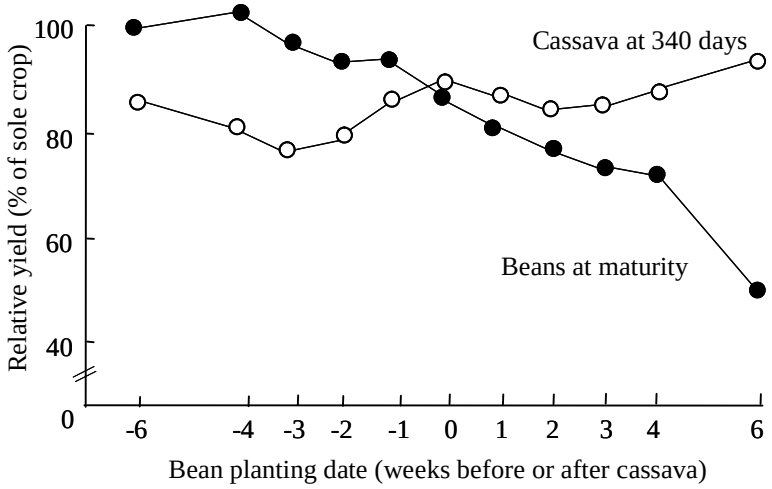

Figure 2. Relative yields of cassava and common beans (Phaseolus vulgaris), grown in association, according to their relative planting times.
***Source**: Thung and Cock, 1979.*

Planting density

In general, the optimum monocrop planting density can also be used when cassava is grown in association with other crops without causing a serious yield reduction of the associated crop. However, if the cassava variety is very vigorous, it may be necessary to reduce its plant density in order to maximize total yields. With late-branching and less vigorous cassava varieties the best yields were achieved with an intermediate plant density of about 10,000 plants per hectare.

Planting pattern

The choice of spatial arrangement of each crop is important in reducing competition and maximizing total yield, as different arrangements affect the efficiency of utilization of light and space. In many cases, a normal square planting arrangement of cassava with one row of grain legume or maize between cassava rows gives the maximum yield and income from both crops. However, to favor the growth of intercrops, a wider interrow spacing of cassava and shorter interplant spacing within the row is often preferred.

This arrangement may allow the planting of two or more rows of intercrops between cassava rows. In Indonesia, cassava is often planted with an interrow spacing of 1.8-2.0 m and interplant spacing of 0.5 m, which allows the planting of 4-5 rows of upland rice or peanut planted between rows in addition to one hill of maize between cassava plants in the row. After the harvest of upland rice and maize, there is still enough light between rows for planting a second intercrop of a short-duration grain legume between cassava rows. Alternatively, cassava can be planted in double rows spaced at 0.8x0.8 m in each double row, with 1.9-2.0 m between double rows. This will allow the planting of several rows of intercrops between each double row of cassava. By varying the interrow and interplant spacing, a cassava plant density of about 10,000 plants/ha can be maintained. Within limits, whether cassava is planted in a square or rectangular planting pattern has little effect on cassava yields (**Table 3**).

Table 3. Effect of various spatial planting arrangements on the yield of cassava at a constant plant density at three locations in Colombia.

Locality	Variety	Spatial arrangement (m)	Density (plants/ha)	Fresh root yield (t/ha)
CIAT-Palmira[1)]	MMex 52	1.0 x 1.0	10,000	25.0
		2.0 x 0.5	10,000	22.0
CIAT-Palmira	MCol 22	1.0 x 1.0	10,000	35.0
		2.0 x 0.5	10,000	37.0
Caribia	MCol 22	1.0 x 1.0	10,000	17.1
		1.8 x 0.6	9,259	17.6
Media Luna	Secundina	1.0 x 1.0	10,000	15.0
		1.6 x 0.6	10,416	14.1

[1)] At CIAT- Palmira, the effect of spatial arrangements on cassava yield was statistically not significant. No statistical analyses were performed for the other two locations.
Source: *CIAT, 1979 and 1980.*

The spacing of the intercrops planted between the cassava rows depends on the growth habit of the crop. Most grain legumes should be planted at least 50-70 cm from the nearest cassava row to prevent excessive competition from cassava. Within the remaining interrow space, 2-3 rows of legumes can be grown at 30-50 cm between rows. Intercropping cassava with common beans at CIAT, the arrangement of three rows of beans (spaced a 30 cm between rows) planted between cassava rows (spaced at 1.8 m between rows) produced the highest total yield and income (**Figure 3**). However, in North Vietnam the planting of two rows of peanut between cassava rows, spaced at 1 m between rows, was most profitable (Le Sy Loi, 2000).

Fertilization

Crops grown in association tend to cause less loss of nutrients through erosion and leaching but more loss of nutrients removed in the harvested products. Intercropping represents an intensification of the demand for nutrients, particularly when each associated crop is planted at its normal density. In this case, the removal of nutrients from the soil is higher than when cassava is grown in monoculture (**Table 4**).

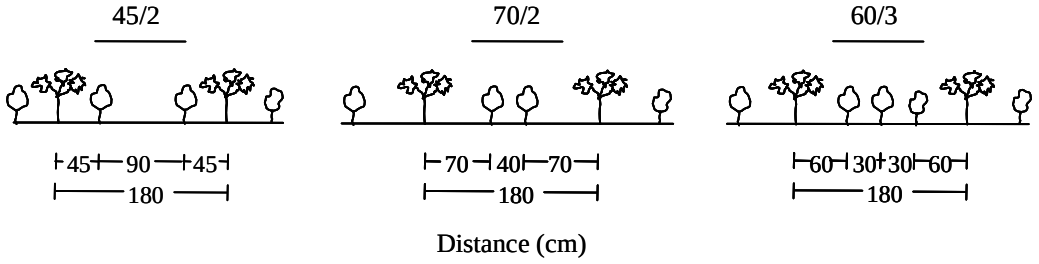

Figure 3. Spatial arrangements for cassava in association with legumes, planted on flat land. ***Source:*** *CIAT, 1979.*

Table 4. Removal of soil nutrients by the products (roots and grains) harvested in a cassava/ mungbean intercropping system, compared to removal by cassava planted in monoculture.

	Nutrients removed (kg/ha)					
System	N	P	K	Ca	Mg	S
Cassava in monoculture	40	5	78	19	8	6
Cassava/mungbean intercropping	90	11	84	18	10	9

There is little or no information about the optimum rates and balance of N, P and K fertilization for each crop in an intercropping system, because this is highly dependent on the fertility of the soil, on the nutritional requirements of each crop, their competitive interaction and growth duration. Whether most fertilizers should be applied to cassava or to the intercrop also depends on the expected income to be derived from each crop. In general, cassava should be fertilized as if it were planted in monoculture, generally requiring relatively high levels of N and K, while cereal crops require mostly N and P, and grain legumes P and K.

Weed control

Intercropping cassava tends to reduce the growth of weeds between cassava rows, but it also makes weeding by mechanical means more difficult. One hand weeding with a hoe at 3-4 weeks after planting is often practiced, after which the canopy cover from both cassava and the intercrops will generally prevent further weed growth.

Weed competition can also be reduced by application of pre-emergence herbicides. However, some herbicides that are selective for cassava may not be selective for the intercrop. Thus, care should be taken in the selection and dosage of the appropriate herbicides, as discussed in Chapter 22.

Evaluation of Intercropping Systems

Intercropping as a production system is adopted both for biological and economic reasons. In subsistence farming systems, most of the agriculture products are consumed

directly on the farm and therefore, biological production is very important. To measure the biological efficiency there are two basic concepts:

- Land Equivalent Ratio (Mead and Willey, 1980)
- Area Time Equivalency Ratio (ATER) (Hiebsch, 1978)

Land Equivalent Ratio (LER)

The Land Equivalent Ratio is a way to assess whether growing crops in association results in a higher yield as compared with growing the two or more crops in separate fields in monoculture. In other words, whether the interaction between the crops grown in association is positive or negative. The LER is calculated using the following equation:

$$LER = \frac{Y(I)_x}{Y(M)_x} + \frac{Y(I)_y}{Y(M)_y} + \cdots\cdots \frac{Y(I)_z}{Y(M)_z}$$

where Y(I)x, Y(I)y and …Y(I)z are the yields of crops x, y and …..z in intercropping

Y(M)x, Y(M)y and …Y(M)z are the yields of crops x, y and….z in monoculture

The LER indicates how much land (in ha) would be needed to grow the various crops in separate fields in monoculture to obtain the same yields of each crop as those obtained in one ha grown in the intercropping system. Thus, the higher the LER, the more productive the intercropping system is as compared to monoculture. The LER is used mainly to compare different intercropping systems, such as different combinations of crops and different management systems. This measure is useful to express and evaluate:

- *Maximum production criterion:* The advantage and disadvantage, in terms of biological production of intercropping as compared to monocropping
- *Least area criterion:* The efficiency or inefficiency of one system as compared to another one with regard to land use
- *Crop combination comparison:* The advantage or disadvantage of one combination of crops over another one.

Area Time Equivalency Ratio (ATER)

Intercropping systems involve intensification in both time and space. Crop production is not solely a function of land area, crop, management, and environment as implied by LER, but it is also related to the duration of crop growth, or the time during which the land is occupied by a crop or crop combination.

The ATER compares the relative productive capacities of the crop in the two systems, indicating which system is more effective in the use of area and time to produce a given quantity of yield. In mathematical terms the ATER is calculated as follows:

$$ATER = \frac{1}{t(I)} \{ t(M)_x \times \frac{Y(I)_x}{Y(M)_x} + t(M)_y \times \frac{Y(I)_y}{Y(M)_y} + \ldots\ldots\ldots t(M)_z \times \frac{Y(I)_z}{Y(M)_z} \}$$

where t (M) is the growing period (days) of crops x, y and……z in monoculture

t (I) is the total time (days) of the intercropping system

Y(I) is the yield (t/ha) of crops x, y and……..z in intercropping

Y(M) is the yield (t/ha) of crops x, y and…… .z in monoculture

Table 5 shows an example of the calculation of LER and ATER for three intercropping systems used in CIAT-Colombia (Leihner, 1983). The LER and ATER for the cassava/bushbean/climbing bean intercropping system are calculated as follows using the experimental data shown in **Table 5**:

LER = 24.8/28.2 + 2.0/2.2 + 0.6/1.5 = 0.879 + 0.909 + 0.355 = 2.188

ATER = 1/395(395 x 24.8/28.2 + 110 x 2.0/2.2 + 106 x 0.6/1.5) = 1/395(347.38 + 100.00 + 42.40) = 1/395 x 489.78 = 1.240

Table 5. Yields of cassava and beans, land equivalent ratio (LER) and area-time equivalency ratio (ATER) in various cropping systems at CIAT-Colombia.

	Yield (t/ha)			Crop duration (days)				
Cropping system	Cassava	Bush b.	Climb. b	Cassava	Bush b.	Climb.b.	LER	ATER
Cassava monoculture	28.2	-	-	395	-	-	-	-
Bush bean monoculture	-	2.2	-	-	110	-	-	-
Climbing bean monocult.	-	-	1.5	-	-	106	-	-
Cassava/bushbean	23.0	2.0	-	395	110	-	1.725	1.069
Cassava/climbing bean	31.4	-	0.5	395	-	106	1.446	1.203
Cassava/bush/climbing b.	24.8	2.0	0.6	395	110	106	2.188	1.240

Source: *adapted from Leihner, 1983.*

Economic Evaluation

In comparing alternative intercropping systems, there are several advantages in assessing productivity differences in value terms as given by market prices, because it is possible to aggregate the different crop outputs and different inputs using a common unit of measure. Also, differences in quality can be taken into account; and the researcher is evaluating different alternatives on the same basis as the farmer who is mainly interested in maximizing his/her net income.

The net income measure is effective in selecting between different cropping systems, especially when there is competition between the associated crops and the issue arises as to whether to increase the relative yield of one crop over the other crop depending on the market price of each crop. The economic evaluation is also particularly useful when there are major differences in input levels and, therefore, in production costs; or when there are differences in the relative value of the crops between regions, which may affect the profitability. When calculating net income, i.e. gross income minus total production costs, it is important to include all variable costs and not just the cost of the production factor under study, such as the cost of fertilizers.

EXPERIMENTAL RESULTS

Many experiments have been conducted to determine the best plant spacing and planting patterns, and comparing different intercrops to identify those that maximize yields and income. Only a few examples are shown below.

1. Intercropping of cassava with grain and forage legumes in Quilichao, Colombia

Cassava, cv. MCol 1684, was intercropped with various grain and forage legumes as shown in **Table 6**; the legumes were planted at the same time as cassava. Common bean and soybean produced low yields due to Al and Mn toxicity, in spite of the application of 0.5 t/ha of dolomitic lime, while peanut produced a reasonable amount of grain. Cassava yields were significantly reduced by intercropping with Stylo (*Stylosanthes guianensis*), which competed strongly for water during the dry season; and with cowpea, and velvet bean, mainly due to competition for light. Competition by cowpea was most pronounced during the first three months after planting, and by velvet bean at 3-5 months. Intercropping with peanut reduced cassava yields only 8%, while producing a considerable amount of grain and crop residues (not shown).

Table 6. Effect of intercropping cassava with grain and forage legumes on the yield of cassava, cv. MCol 1684, and intercrops when grown in CIAT-Quilichao, Colombia.

Intercrop treatments	Intercrop yield (kg/ha)	Cassava root yield (t/ha)
1. Cassava monoculture	-	38.4 a
2. Cassava + cowpea	338 [1)]	25.2 b
3. Cassava + common bean	104 [1)]	37.1 a
4. Cassava + peanut	609 [1)]	35.2 a
5. Cassava + soybean	21 [1)]	31.1 ab
6. Cassava + velvet bean	1,153	26.6 b
7. Cassava + *Stylosanthes guianenssis*	4,748	26.5 b
8. Cassava + *Pueraria phaseoloides*	2,079	30.8 ab
F-test		**

[1)] dry grain only

2. Intercropping cassava with maize and several legumes in Vietnam

In Vietnam many small-holder farmers intercrop cassava to maximize their food production or income from a small area of land. An intercropping trial was therefore conducted at Hung Loc Agric. Research Center in South Vietnam to determine the best intercrop and planting arrangement for this system. **Figure 4** shows that the single row planting of cassava at 1.0 x 1.0 m produced higher cassava yields and net profits than the double row system for all intercrops except maize. All intercrops reduced cassava yields, especially intercropping with the long-duration *Canavalia ensiformis*. Net profits were also highest for planting cassava in monoculture, while among the intercrops peanut produced the highest total net profit in both the single and double row systems. Peanuts were also the most productive intercrop in several experiments conducted in North Vietnam, especially when planting two rows of peanut between single rows of cassava spaced at 1 m (Le Sy Loi, 2000; Trinh Phuong Loan, personal communication)

3. Intercropping cassava with cereal and legume crops in Indonesia

Farm size in Indonesia is extremely small while labor is quite abundant in most areas, especially on Java island. For that reason most cassava is grown with at least one and sometimes up to four intercrops in order to maximize food production to feed the family and for sale. In southern Sumatra where rainfall is rather abundant with only a 3-4 month dry season, farmers often intercrop cassava with upland rice between cassava rows

and maize between cassava plants in the row. After the harvest of rice and maize at 3-4 month after planting (MAP), they may plant peanut or mungbean between cassava rows, followed by cowpea if rainfall permits. In East Java where the dry season extends to 5-6 months, cassava is generally only intercropped with maize, planted in single rows along side the cassava rows.

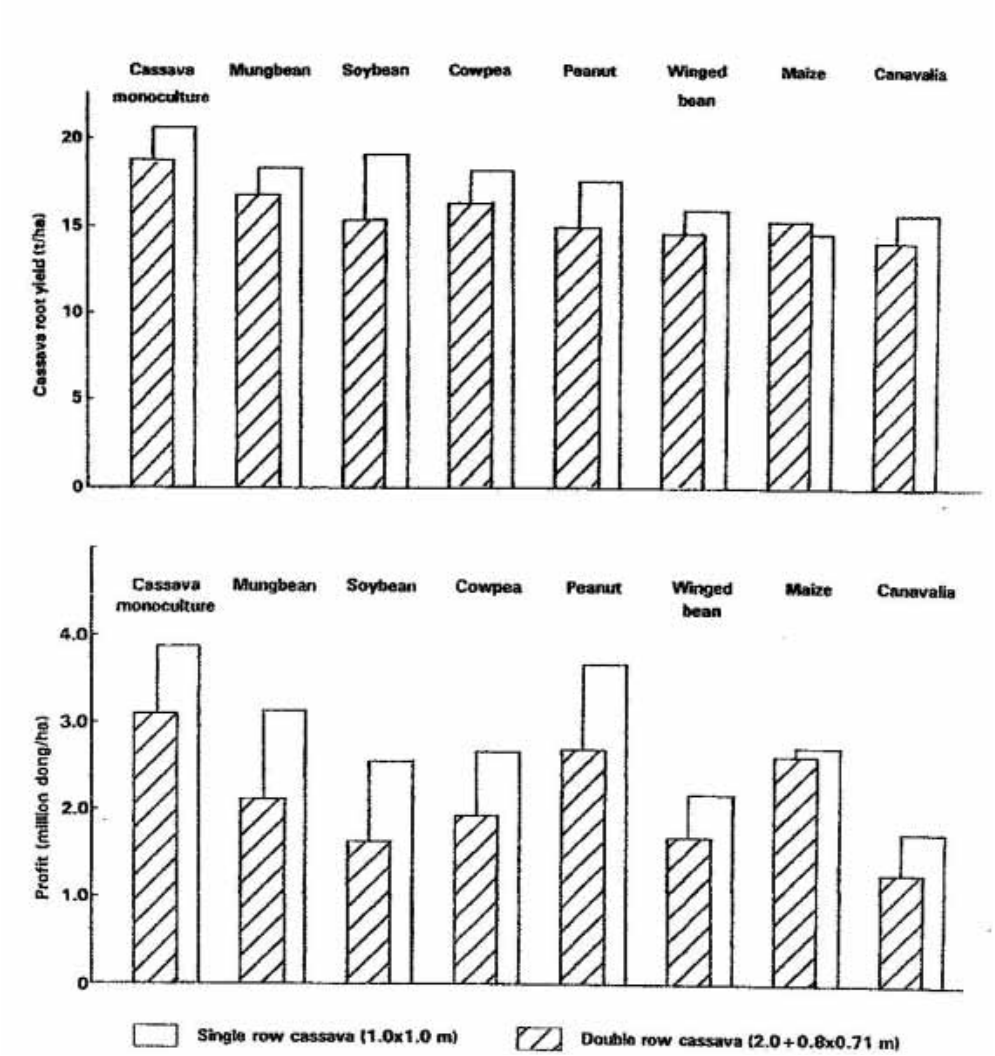

Figure 4. Average effect of various intercropping systems on cassava yields (top) and on total net profits (bottom) when cassava was planted in single rows at 1.0 x 1.0 m or in double rows at 2.0 x 0.8 x 0.71 m at Hung Loc Center in South Vietnam in 1989, 1990, 1991 and 1992. ***Source:*** *Nguyen Huu Hy et al., 1995.*

An intercropping experiment was conducted from 1987 to 1990 in Tamanbogo in Lampung province of Sumatra to determine the best plant spacing and planting pattern of cassava when intercropped with various combinations of 3-4 crops, i.e. rice, maize, peanut, mungbean and cowpea. Cassava was planted at three spacings, i.e. 1.0 x 1.0 m, 2.0 x 0.5 m

and the double-row system of 2.73 x 0.6 x 0.6 m, which all result in a cassava plant population of 10,000 plants/ha. Upland rice and maize were planted at the same time as cassava in the early part of the rainy season (Oct/Nov), while peanut or mungbean were planted between cassava rows after the harvest of rice, and cowpea was planted after the harvest of the peanut/mungbean crop. **Table 7** shows the contribution of cassava and the first, second and third intercrops to the total gross income in each cropping system.

Table 7. The effect of cassava cropping system and planting arrangement on the total gross income and the relative contribution of each crop to total gross income in Tamanbogo, Lampung, Indonesia. Data are average values for three years (1987-1990).

Cassava plant spacing/ Cropping system[1)]	Total gross income ('000 Rp/ha)	Relative contribution to income (%)			
		First intercrop(s)	Second intercrop	Third intercrop	Cassava
1.0 x 1.0 m					
Cassava monoculture	1,386	-	-	-	100
C+M+R-P-CP	1,466	37	14	2	47
C+M-P-CP	1,406	25	19	3	53
C+R-P-CP	1,581	32	15	3	50
C+P-MP-CP	1,577	35	6	3	56
2.0 x 0.5 m					
Cassava monoculture	1,242	-	-	-	100
C+M+R-P-CP	1,550	36	18	3	42
C+M-P-CP	1,378	23	23	3	51
C+R-P-CP	1,607	33	17	3	47
C+P-MP-CP	1,464	35	5	3	57
2.73 x 0.6 x 0.6 m					
Cassava monoculture	1,240	-	-	-	100
C+M+R-P-CP	1,486	39	13	3	45
C+M-P-CP	1,299	25	21	3	51
C+R-P-CP	1,477	27	17	2	53
C+P-MP-CP	1,666	32	6	4	58

[1)] C = cassava, M = maize, R = upland rice, P = peanut, MB = mungbean, CP = cowpea
Source: *Wargiono et al., 1995*.

When planted in monoculture, cassava obviously contributed 100% to the total gross income. In monoculture highest yields were obtained with the square planting arrangement of 1.0 x 1.0 m, while there was no difference in yield between the wide-row spacing and the double-row arrangement. When intercropped, cassava contributed only about 45-58% to the total gross income, while the remaining income came from the various intercrops. Averaged over the three planting patterns, the system of cassava intercropped with peanut followed by mungbean and cowpea produced the highest gross income. Averaged over the four intercropping systems the highest gross income was obtained with the square planting pattern (1.0 x 1.0 m), which was slightly higher than that obtained with the wide-row spacing (2.0 x 0.5 m), while the double-row spacing produced the lowest

gross income (Wargiono *et al*., 1995). In a similar trial conducted in Yokyakarta in 1987 and 1988 the square planting at 1.0 x 1.0 m again produced the highest total crop value in two intercropping systems (Wargiono *et al.*, 1992).

From these various experiments it may be concluded that intercropping tends to decrease cassava yields, but the yield of the intercrops will often more than compensate for the loss in cassava yield, and will provide the farmers with additional food or cash long before cassava is ready for harvest. However, unless the intercrops are well-fertilized, incorporation of their residues will generally have little long-term effect on soil fertility. Among the various intercrops tested, peanut seems to be most compatible with cassava as it can grow in relatively acid and low fertility soils and does not compete as much with cassava as most other crops. Upland rice and maize are also successfully intercropped with cassava, mainly in Indonesia.

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CHAPTER 24

CASSAVA LEAF PRODUCTION FOR ANIMAL FEEDING [1]

Reinhardt Howeler[2]

INTRODUCTION

Cassava is generally grown for the production of its roots, which are high in starch but low in protein. Before the roots are harvested the top growth is cut off, from which the best stems are selected as planting material for the next crop. The remaining leaves and stems are generally left on the ground and incorporated in the soil during land preparation before the next planting. However, these leaves are high in protein and could be utilized for human consumption or animal feeding. In some countries the leaves and young green tops remaining after the root harvest are taken home to be fed to animals. The green tops are a good source of protein, especially for ruminants which can digest the rather high fiber content of the green stems and petioles. The upper young leaf blades may contain as much as 30% crude protein, while the green tops usually contain 20-25% protein, which is higher than most forage legumes. Thus, there is a great potential for using cassava tops as supplemental forage for animals, or young cassava leaves for human consumption. The leaves or young tops can be harvested either only once at time of root harvest, or they can be cut several times, about every 2½-3 months. After cutting off the tops, the remaining stem will resprout to produce new shoots, which can be cut again 3-4 times in a one-year crop cycle, or 6-7 times during a two-year crop cycle. These young plant tops tend to have a higher protein and lower fiber content than leaves harvested only once at time of root harvest.

Since cassava leaves have a high concentration of cyanide, which is toxic when consumed, the leaves need to be wilted at least overnight by spreading on the floor to reduce the cyanide content by evaporation. Sun- or oven-drying is even more effective in reducing cyanide, while ensiling for 90 days will lower the cyanide content to only 10-15% of its initial value (Nguyen Thi Hoa Ly *et al*., 2010). The dry or ensiled cassava leaves can also be stored for several months without spoiling.

Cassava Leaf Production Experiments

To determine the dry matter and protein production potential of cassava foliage[3], as well as the effect of repeated leaf harvests on root yields, various experiments were conducted to select the most productive cassava varieties, fertilization, plant spacing, and frequency and height of cutting, both in Colombia in the 1980s as well as in Thailand, Vietnam, China and Indonesia in the early 2000s. Some of these are briefly described below.

One experiment on varieties and time of fertilizer application was conducted in CIAT-Quilichao, Colombia, located at about 1000 masl.. The treatments consisted of 16 varieties or breeding lines, ten of which were well adapted to year-round cold climates at

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[1] This chapter has borrowed extensively from the paper by Chalaem Martwana *et al*., 2009.

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[3] In this chapter "leaves", "foliage" or "forage" usually refer to the whole green tops including leaf blades, petioles and upper green stem.

high elevations of 1500-2000 masl, and the remaining six varieties were adapted to warmer climates at low- or mid-altitudes of <1000 masl. All plants were fertilized with 1 t/ha of 10-20-20, applied either all at planting or fractionated with 40% at planting and 20% after each of the first three cuts.

Table 1 shows the total amount of dry forage produced in four cuts (the last corresponding to the final root harvest), as well as the yield of fresh roots harvested at 14 months after planting (MAP). Total dry forage production varied from 7.5 to 13.9 t/ha. Varieties adapted to cold climates had a more vigorous top growth and resprouted better than those from the warmer climate, as indicated by the average dry forage production of 11.6 t/ha for the former and 8.8 t/ha for the latter. However, those varieties producing much forage tended to have low root yields and *vice versa.* Thus, varieties can be selected that are mainly producers of tops (CG 358-3), those that produce mainly roots (CM 489-1), and those that produce relatively high yields of both tops and roots (Regional Negrita and CM 849-1). When all fertilizers were applied at planting, forage yields were highest in the first two cuts, but much lower in the last two cuts compared with the fractionated fertilization treatment. Total forage yields were not significantly different between these two treatments (**Table 2**).

Table 1. Total dry cassava forage production and its N and crude protein content of 16 varieties and breeding lines obtained in four cuts during 14 months in Quilichao, Colombia. Root production corresponds with the final harvest of the whole plant.

			In forage	
Variety [1)]	Dry forage [2)] (t/ha)	Fresh roots (t/ha)	N (kg/ha)	Crude protein (t/ha)
1. CMC 92	11.7	8.9	378	2.4
2. Regional Amarilla	12.3	18.8	345	2.1
3. Regional Negrita	11.5	28.2	366	2.3
4. Americana	10.6	13.8	338	2.1
5. Algodona	13.0	11.5	394	2.5
6. Sececcion 40	12.0	20.6	355	2.2
7. Batata	11.1	12.7	353	2.2
8. MCol 2016	9.8	20.6	326	2.0
9. MCol 2019	10.1	13.7	326	2.0
10. CG 358-3	13.9	13.1	351	2.2
11. HMC 1	8.4	28.4	298	1.9
12. CM 489-1	7.5	38.0	244	1.5
13. CM 430-37	9.4	17.5	269	1.7
14. MVen 218	10.9	12.4	333	2.1
15. CM 507-37	7.6	27.7	260	1.6
16. CM 849-1	9.3	37.4	277	1.7
Average 1-10	11.6	16.2	353	2.2
Average 11-16	8.8	26.9	280	1.7

[1)] Varieties 1-10 are from cold climates, while varieties 11-16 are from warmer climates

[2)] Sum of four cuts; 1 t/ha 10-20-20 was applied all at planting

Source: *CIAT, 1988b.*

Table 2. Effect of time of application of 1 t/ha of 10-20-20 fertilizers on total dry forage production, crude protein concentration and N content from four cuts of cassava tops. Data are average values of 16 varieties grown in Quilichao.

	Fert.[1)]	First cut at 4 MAP	Second cut at 7 MAP	Third cut at 10 MAP	Fourth cut at 14 MAP	Total of four cuts
Total dry forage (t/ha)	-A	3.75	3.39	1.90	1.47	10.56
	-B	3.20	2.92	2.30	1.79	10.20
Crude protein (%)	-A	20.2	20.1	18.8	17.2	19.3
	-B	18.8	21.3	17.4	15.8	18.5
N in forage (kg/ha)	-A	121	109	57	40	326
	-B	97	99	62	44	302

[1)] A = 100% of 10-20-20 applied at planting; B = 40% at planting and 20% after each cut.

Thus, forage yields are related to fertilization and decline when the soil becomes exhausted, mainly of N. Average N extraction in tops was 326 kg/ha, compared with 133 kg K and 31 kg P/ha. Extraction in the root harvest was approximately 25 kg N, 26 kg K and 7 kg P/ha. The chemical fertilizer supplied about 100 kg N, part of which would probably be lost through leaching. Thus, of the total amount of 350 kg N extracted by the crop, about 250-300 kg N must come from either the soil organic matter or from other sources. At planting the soil contained 6.8% organic matter (OM) and approximately 5000 kg of total N/ha. If the soil were mineralizing N at a high rate of 3% per year, it would produce only 175 kg N/ha in 14 months. The remaining 75-125 kg N absorbed by cassava in this experiment could have come from N in rain water (usually no more that 20-30 kg/ha) or through N fixation by association with N-fixing bacteria, either on the roots or on the leaf surfaces. This should be further investigated.

Another experiment was conducted to determine in more detail the best combination of N, P and K to optimize either leaf production, root production or both. The experiment was conducted on plots that had previously been planted for three years to study the effect of different combinations N, P and K on root production. In this experiment the same treatments were again established on the same plots of 7.2 x 5.4 m. Three varieties were planted in each plot. In one half of these subplots, cassava tops were cut at 3, 8½ and 12½ MAP, while plant tops were not cut in the other half. Stakes were planted at a distance of 40 x 45 cm for a plant population of 55,555 plants/ha. The soil was quite acid (pH 4.2-4.4), had a high OM content (6.8-7.2%), but was very low in available P and exchangeable K.

Table 3 shows the effect of the various N, P and K application rates on the yields of dry forage and fresh roots in those plots where plant tops had been cut three times during the 12½ month growth cycle, as well as the fresh root yields of plants that did not have any top pruning. Without any fertilizer applied the total dry forage yield was only 2.8 t/ha, while the corresponding fresh root yield was 11.7 t/ha; without leaf pruning the root yield nearly doubled to 22.2 t/ha. Both the forage and root yields increased markedly with application of 50 kg N, P or K. Higher rates of application of N or P did not further increase forage or root yields, but higher rates of K did increase both forage and the corresponding root yields; however, the increase in root yield was much more pronounced in plants without top pruning. At the highest rate of fertilization of 200 kg/ha of N, P and K

the pruned plants produced 7.27 t/ha of dry forage and 18.3 t/ha of fresh roots, while the unpruned plants produced 30.5 t/ha fresh roots plus the forage remaining on the plants at time of root harvest, which was not determined. While top pruning produced a considerable amount of high protein forage, this practice also reduced root yields on average about 40%

Table 3. Effect of annual applications of various combinations of N, P and K on the average dry forage and fresh root yields of three cassava varieties[1)] planted for both root and leaf production in Quilichao, Colombia in 1983/84.

	With three cuts of tops		Without top cutting
Treatments [2)]	Dry forage (t/ha)	Fresh root yield (t/ha)	Fresh root yield (t/ha)
$N_0P_0K_0$	2.80	11.7	22.2
$N_0P_2K_2$	3.57	14.5	25.8
$N_1P_2K_2$	6.43	16.3	28.9
$N_2P_2K_2$	6.22	15.0	27.6
$N_3P_2K_2$	6.43	17.6	25.1
$N_2P_0K_2$	3.34	11.5	23.9
$N_2P_1K_2$	6.20	19.6	27.7
$N_2P_3K_2$	6.96	19.0	30.1
$N_2P_2K_0$	3.27	8.6	13.9
$N_2P_2K_1$	5.46	18.8	25.7
$N_2P_2K_3$	7.75	19.6	29.8
$N_3P_3K_3$	7.27	18.3	30.5
Average	**5.48**	**15.9**	**25.9**

[1)] Varieties: CM 523-6, CM 489-1 and CM 91-3

[2)] N_0 = 0N P_0 = 0P K_0 = 0K
N_1 = 50N P_1 = 50P K_1 = 50K
N_2 = 100N P_2 = 100P K_2 = 100K
N_3 = 200N P_3 = 200P K_3 = 200K

Finally, another experiment was conducted in both Carimagua and Quilichao in Colombia using four well-adapted varieties, which were planted at four different plant spacings: 70 x 70, 60 x 60, 50 x 50 and 40 x 40 cm, corresponding to populations of 20.4, 27.8, 40.0 and 62.5 thousand plants/ha. Plants were fertilized with 1 t/ha of 15-15-15 fertilizers, band applied at planting. The unlignified part of the tops were cut every 3-4 months, or when the bottom leaves started to drop off.

Table 4 shows the total dry forage and crude protein produced from three cuts during a 13 month growth period in Carimagua. Of the four varieties, CM 507-37 produced the highest average dry forage yield of 4.3 t/ha, with a crude protein production of 0.89 t/ha. The average crude protein content decreased from 25% in the first cut to 21% in the third cut. Due to the long dry season in Carimagua there was a six month interval between the second and third cut. The best planting distance was 60 x 60 cm, and there was no beneficial effect in further increasing the plant population.

In Quilichao the experimental design was the same but the varieties were different from those used in Carimagua. Two varieties, Regional Amarillo and CMC 92, are adapted to cooler climates at elevations up to 2000 masl, while the other two varieties, HMC 2 and

CMC 40, are more adapted to warmer climates. **Table 5** shows the effect of plant spacing on both root and forage production.

Table 4. Effect of planting distance on the total dry forage (A) and crude protein (B) production from three cuts of cassava tops of four varieties planted in Carimagua, Colombia, during a 13 month growth cycle.

A. Dry forage production (t/ha)

	Planting distance (cm)				
Varieties	70 x 70	60 x 60	50 x 50	40 x 40	**Average**
CM 407-34	2.14	3.30	3.80	3.70	**3.23**
CM 507-37	4.34	4.31	4.38	4.16	**4.30**
CM 723-3	3.38	3.73	2.94	4.83	**3.72**
CM 996-6	2.53	3.77	2.78	2.49	**2.89**
Average	**3.10**	**3.78**	**3.47**	**3.79**	**3.53**

B. Crude protein production (kg/ha)

	Planting distance (cm)				
Varieties	70 x 70	60 x 60	50 x 50	40 x 40	**Average**
CM 407-34	471	758	936	741	**724**
CM 507-37	847	960	925	841	**893**
CM 723-3	730	914	723	1,097	**866**
CM 996-6	576	962	706	555	**700**
Average	**653**	**898**	**822**	**808**	**796**

Table 5. Effect of planting distance on the total dry forage (A) and fresh root (B) production after seven cuts of cassava tops of four varieties planted in Quilichao, Colombia, during a 24 month growth cycle.

A. Dry forage production (t/ha)

	Planting distance (cm)				
Varieties	70 x 70	60 x 60	50 x 50	40 x 40	**Average**
Regional Amarilla	20.74	23.76	20.85	21.07	**21.60**
CMC 92	19.39	22.95	20.34	20.16	**20.71**
HMC 2	14.40	15.52	15.41	16.66	**15.50**
CMC 40	10.10	12.64	11.08	12.39	**11.55**
Average	**16.16**	**18.72**	**16.92**	**17.57**	**17.34**

B. Fresh root yields (t/ha)

	Planting distance (cm)				
Varieties	70 x 70	60 x 60	50 x 50	40 x 40	**Average**
Regional Amarilla	27.8	28.4	23.7	19.8	**24.9**
CMC 92	29.8	23.9	22.4	18.9	**23.8**
HMC 2	45.3	52.0	46.0	41.8	**46.3**
CMC 40	31.8	36.1	23.9	28.4	**32.7**
Average	**33.7**	**35.1**	**32.2**	**26.7**	**31.9**

Source: *CIAT, 1988a*.

Highest total dry forage production of nearly 24 t/ha was obtained with the variety Regional Amarillo, planted at 60 x 60 cm, closely followed by CMC 92 at the same planting distance. Both are cold climate varieties which produce extremely vigorous top growth when planted at lower elevations. Much less productive were the warm-climate varieties, HMC 2 and CMC 40, which did not have the same capacity to resprout quickly after each cut. However, those varieties less productive in forage production were more productive in terms of root yield, especially HMC 2, which produced as much as 52 t/ha fresh roots as well as 16 t/ha of dry forage in two years.

Figure 1 shows that the quantity and quality of forage production is highly dependent on rainfall. During the dry seasons, dry matter production decreased while the crude protein concentration increased; the opposite occurred during the wet seasons. Only during the last cut was there both a decrease in dry matter production and protein concentration.

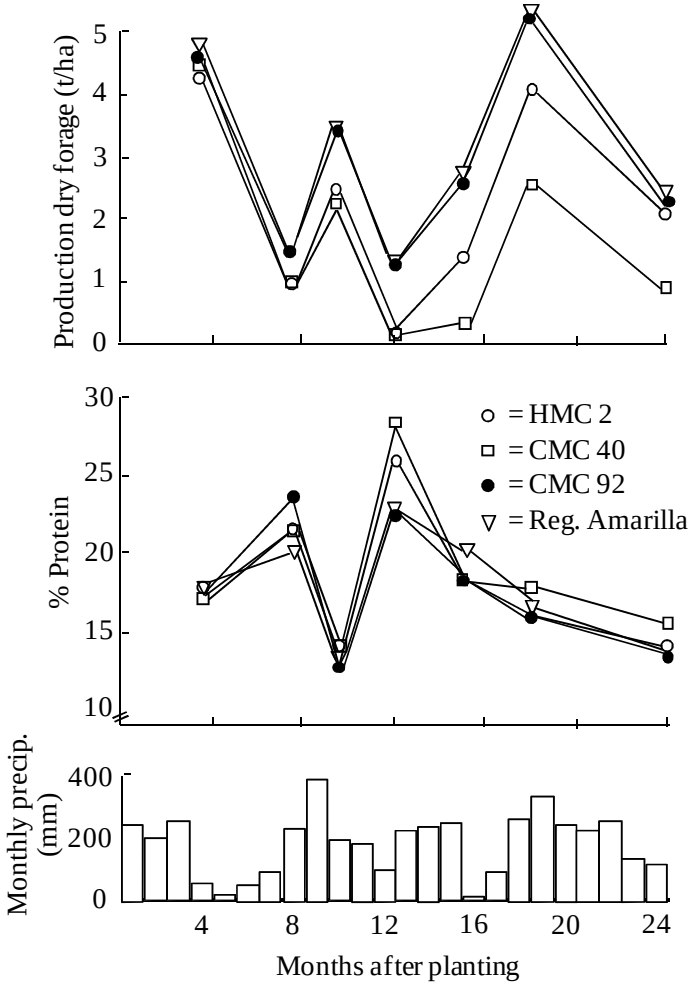

Figure 1. Dry weight and crude protein content of cassava forage from four varieties in each of seven cuts as influenced by seasonal fluctuations of precipitation during a two year period in Quilichao, Colombia. Data are the average values for four plant spacings.
***Source**: CIAT, 1988a.*

Thus, while the dry matter production varied according to wet and dry seasons, the amount of crude protein produced was fairly constant throughout the year. Total crude protein production varied from 2.0 t/ha with CMC 40 to 3.67 t/ha for Regional Amarilla in a 2-year period. The latter level of productivity is close to that of well-managed alfalfa in the US. While this trial was harvested after two years, there is no reason to believe that forage production can not continue for one or more years without having to replant. With the application of 1 t/ha of 15-15-15 at planting, there was no indication of soil exhaustion, as the soil nutrient levels had actually increased after two years.

From these results it is clear that varieties differ greatly in their ability to withstand continuous cuttings, and that some cold-climate varieties seem very promising for cassava forage production when planted at lower elevations or in warmer climates. A plant population of 28,000 plants/ha, planted at 60 x 60 cm, was the most promising in both Carimagua and Quilichao. No replanting may be necessary for several years.

More recent research results from Colombia indicate that a plant spacing of 30 x 30 cm (111,000 plants/ha) resulted in the highest fresh leaf production of 91 t/ha after 12 months (Ospina *et al*., 2007). At another location, the fresh leaf yield was 98 t/ha with three cuttings in nine months, using raised beds and 70 x 30 cm planting spacing or 48,000 plants/ha (**Table 6)**. The N removed from the soil for each ton of fresh foliage was 7.3 kg, which was double that of N removed in the root harvest.

Table 6. Production and quality of fresh foliage, as well as the nutrients removed in the foliage of cassava, cv. HMC 1, planted at 70 x 30 cm during an 11 month growth cycle in Candelaria, Valle de Cauca, Colombia.

Age at cutting	Fresh foliage yield (t/ha)	Crude protein content (%)	Crude fiber content (%)	Fat content (%)	Nutrients removed (kg/ha)					
					N	P	K	Ca	Mg	S
3 months	18.0	26.7	29.6	5.5	231	17.3	123	92	25.4	15.7
7 months	53.5	18.3	32.0	4.8	308	37.9	210	132	44.2	25.3
9 months	26.7	20.5	25.9	4.3	178	19.8	100	77	29.7	13.2
Total	**98.2**				**717**	**75.0**	**433**	**301**	**99.3**	**54.2**
Average extraction per ton fresh forage harvested:					7.30	0.76	4.41	3.07	1.01	0.55

***Source**: Ospina et al., 2007.*

In Vietnam, of the two plant populations studied, the population of 22,222 plants/ha with a plant spacing of 90 x 45 cm produced the highest cassava dry leaf yield (Nguyen Huu Hy *et al*., 2007).

Experiments conducted in China showed that the harvesting of young shoots at 4-8 months after planting significantly reduced the cassava root yield. The earlier the leaves were harvested, the lower the root yield (Li Kaimian *et al*., 2007).

Similar experiments conducted in Thailand indicate that the dry leaf yield ranged between 3-8 t/ha with a protein content of 12-34%, while the root yields and the starch contents in the root were 7-23 t/ha and 11-29%, respectively (Limsila *et al*., 2007)

Further intensive research on cassava leaf production technologies for animal feed was conducted in two locations in Thailand, at the Rayong Field Crops Research Center (FCRC) and at the Khon Kaen FCRC, both under the Department of Agriculture, during 2002/03 and 2003/04 (Martwanna *et al*., 2009).

The soils at both Centers are light textured, at Rayong a sandy clay loam and in Khon Kaen a sandy loam, with less than 1% organic matter and low levels of Ca, Mg and K, and relatively high levels of P due to previous applications of P fertilizers. The climate is a tropical monsoon with year-round high temperatures (mean 26-28ºC) and relatively high and unpredictable rainfall of 1,000-1,400 mm/year, falling mostly between June and October.

Four experiments were conducted at both sites to determine the leaf yield potential of different cassava varieties, the optimum NPK fertilization, plant spacing as well as the height and frequency of cutting. In all four experiments cassava stakes were planted vertically at 30 x 30 cm (except in the plant population experiment). Plants were fertilized with a total of 520 kg N, 150 P_2O_5 and 150 K_2O/ha (except in the fertilizer experiment), applied fractionated at planting and after each cut. At 2½-3 month intervals the green stems with leaves and petioles were cut off at about 20 cm above the ground (except in the cutting height and frequency experiment). These green shoots were weighed and then chopped by a mechanical chopper; a sample from each plot was sun- and oven-dried and weighed again to determine the dry matter content, and a subsample was analyzed for N to determine the protein concentration and yield. In most cases green tops were cut at about 2½, 4½, 6½, 10 and 12 months after planting, the last cut coinciding with the root harvest.

The various treatments in these experiments are described in more detail below.

Variety experiment for leaf production

The variety experiment consisted of 24 varieties and breeding lines. The varieties were the standard varieties released for their high root yields by the Department of Agriculture, while the breeding lines had been selected for their vigorous top growth.

Cassava leaf and root yields, and the starch content of roots were higher in Rayong than in Khon Kaen during both years. In Rayong the dry leaf and fresh root yields and starch contents in 2002/03 were higher than in 2003/04. This was due to a good rainfall distribution in the early and late rainy season in 2002/03. In 2003/04, although the total amount of rainfall was similar to that of 2002/03, there were dry periods before planting, during early plant growth and later in the season. In 2002/03 CMR 42-07-09, CMR 41-33-34 and CMR 42-59-173 had the highest dry leaf yields. However, the standard varieties, Rayong 90, Rayong 5 and Rayong 72 produced the highest root yields. The lines CMR 42-90-338 and 42-01-2 had consistently the highest root starch contents of 25.2 and 20.2%, and 24.3 and 24.4% for 2002/03 and 2003/04, respectively.

At Khon Kaen, the leaf and root yields showed different trends during the two years. The mean leaf yield was higher in 2002/03 than in 2003/04. However, the root yields showed the opposite trend. For both years, the recommended varieties for root production i.e. Huay Bong 60, KU 50, Rayong 72 and Rayong 90 produced reasonably high leaf and root yields. CMR 41-111-129 as well as Huay Bong 60 produced the highest leaf and root yields in 2002/03 and 2003/04, respectively.

When averaged across locations and years, the dry leaf yields of different cassava varieties/lines ranged from 9.60 to 13.70 t/ha (**Table 7**). CMR 41-61-59, CMR 41-111-129

and Kasetsart 50 (KU 50) produced high dry leaf yields of 13.70, 13.46 and 13.42 t/ha, respectively. The line CMR 41-60-24 had the highest protein content of 19.62%. However, the highest root yields were usually obtained with the varieties Rayong 72, Rayong 90, Huay Bong 60 and KU 50 and the line CMR 41-111-129. CMR 35-22-196 and KU 50 had the highest root starch contents (Martwanna *et al*., 2009).

Table 7. Average results of cassava variety trials for leaf production conducted at Rayong and Khon Kaen FCRC in Thailand during 2002/03 and 2003/04.

	Total dry leaf yield[1)] (t/ha)	Average protein content (%)	Leaf protein yield[1)] (t/ha)	Fresh root yield (t/ha)	Root starch content (%)	Gross income[2)]	Production costs[3)]	Net income
						('000 baht/ha)		
1. Rayong 1	13.23	15.95	2.11	15.52	11.25	63.44	58.63	4.82
2. Rayong 5	11.16	18.71	2.09	17.72	18.05	67.12	56.72	10.40
3. Rayong 60	9.60	18.33	1.76	18.10	12.51	57.63	55.07	2.56
4. Rayong 90	11.27	18.46	2.08	23.70	19.74	73.50	57.76	15.64
5. Rayong 72	11.22	18.22	2.05	24.32	18.10	72.47	57.92	14.55
6. KU 50	13.42	16.90	2.27	20.54	20.49	75.16	59.69	15.47
7. OMR 41-23-41	12.51	17.55	2.20	15.78	15.82	67.14	57.88	9.26
8. CMR 41-42-3	12.45	18.76	2.34	18.54	19.87	74.53	58.28	16.25
9. CMR 41-60-24	11.64	19.62	2.28	16.85	19.38	71.42	57.10	14.32
10. CMR 41-61-59	13.70	17.05	2.34	18.81	12.54	72.04	59.70	12.34
11. CMR 41-111-129	13.46	17.72	2.39	20.46	16.81	76.39	59.72	16.67
12. CMR 41-114-125	10.67	17.60	1.88	13.95	17.31	58.26	55.54	2.72
13. CMR 35-22-196	11.29	18.50	2.09	17.82	22.02	68.64	56.88	11.76
14. CMR 41-20-58	11.96	17.65	2.11	10.93	19.40	61.44	56.45	4.99
15. CMR 41-96-2	10.71	16.44	1.76	11.20	14.80	52.28	55.12	-2.84
16. OMR 41-33-34	13.19	16.11	2.13	12.23	16.36	62.33	58.02	4.31
17. CMR 42-01-2	12.19	16.35	1.99	12.25	16.85	59.30	56.92	2.37
18. CMR 42-07-9	13.09	16.65	2.18	12.62	13.01	63.18	57.98	5.19
19. CMR 42-54-53	10.82	17.93	1.94	14.78	18.34	60.85	55.86	5.00
20. CMR 42-59-173	11.72	18.19	2.13	6.99	15.40	57.52	55.52	2.00
21. CMR 42-61-108	11.09	18.19	2.02	8.37	15.01	55.95	55.06	0.89
22. CMR 42-87-318	11.50	15.91	1.83	14.44	12.97	56.33	56.54	-0.22
23. CMR42-90-338	12.82	15.71	2.02	11.86	19.24	60.03	57.56	2.48
24. Huay Bong 60	12.06	17.77	2.14	21.56	18.55	72.35	58.36	13.99
Average	**11.95**	**17.51**	**2.09**	**15.80**	**16.82**	**64.97**	**57.27**	**7.71**

[1)] Sum of 4-5 cuts

[2)] Prices: cassava roots: 1.2 baht/kg at 30% starch with 0.02 baht reduction per 1% starch reduction
cassava leaves: 24 baht/kg crude protein

[3)] Costs:

15-15-15 fertilizers	baht 520/50 kg	stakes (0.09 baht/stake)	baht 1,600/rai
Urea	430/50 kg	planting (0.045 baht/stake)	800/rai
3 applications of 80 kg/rai of 15-15-15	2,496/rai	weeding	600/rai
2 applications of 35 kg/rai of urea	602/rai	harvesting + chopping	
land preparation	330/rai	+ drying leaves	1,100/t dry leaves
fertilizer application	200/rai	harvesting + transport roots	170/t fresh roots

Note: 1 ha = 6.25 rai; 1 US$ is 40 baht in 2003.

Production costs of all varieties/lines tested ranged from 55,060 to 59,720 baht/ha. The gross incomes from the sale of both roots and dry leaves ranged from 52,280 to 76,390 baht/ha. Twenty two varieties or lines produced a positive net income ranging from 890 to 16,670 baht/ha. The best cassava line with the highest total productivity and net income was CMR 41-111-129 with the second highest dry leaf yield, fourth highest fresh root yield and highest net income. KU 50, Rayong 5, Rayong 72 and Rayong 90 varieties produced good dry leaf and fresh root yields and economic benefits ranging between 11-13 and 17-24 t/ha and 10,400-15,640 baht/ha, respectively.

Fertilization experiment for leaf production

The fertilizer experiment had 12 treatments with various combinations of four levels of N, P and K in main plots and two cassava varieties, Rayong 72 and Rayong 5, in subplots. The treatments were arranged in a split plot design with 4 replications.

Table 8 shows that averaged across varieties, locations and years, the highest leaf yields were obtained with treatments $N_3P_3K_3$ and $N_3P_2K_2$, i.e. 600 kg N combined with 150 or 300 kg P_2O_5 and 150 or 300 kg K_2O/ha. However, high root yields and starch contents were obtained at lower rates of N, P and K, i.e. at $N_3P_2K_2$ and $N_0P_0K_0$, respectively.

Table 8. Average results of cassava fertilizer trials for leaf production conducted at Rayong and Khon Kaen FCRC in Thailand during 2002/03 and 2003/04. Data are average values for Rayong 5 and Rayong 72.

Treatments[1)]	Total dry leaf yield[2)] (t/ha)	Average protein content (%)	Leaf protein yield[2)] (t/ha)	Fresh root yield (t/ha)	Root starch content (%)	Gross income[3)]	Production costs[4)]	Net income
						(000 baht/ha)		
1. $N_0P_0K_0$	3.37	17.94	0.60	10.88	19.29	25.13	26.36	-1.24
2. $N_0P_2K_2$	3.89	17.18	0.66	11.85	18.82	27.47	38.38	-10.92
3. $N_1P_2K_2$	5.65	17.17	0.97	14.36	18.21	37.01	43.68	-6.67
4. $N_2P_2K_2$	8.17	17.77	1.42	20.55	16.69	53.21	50.44	2.77
5. $N_3P_2K_2$	10.16	18.99	1.90	22.25	15.40	65.80	58.79	7.01
6. $N_2P_0K_2$	7.66	17.81	1.35	18.77	17.01	49.93	41.74	8.19
7. $N_2P_1K_2$	7.94	16.97	1.33	20.14	17.36	50.88	46.20	4.68
8. $N_2P_3K_2$	8.44	17.62	1.47	20.01	16.96	54.01	58.48	-4.47
9. $N_2P_2K_0$	6.60	18.86	1.22	16.47	16.24	44.39	45.82	-1.43
10. $N_2P_2K_1$	7.87	17.58	1.35	20.50	17.87	52.02	48.99	3.03
11. $N_2P_2K_3$	7.97	17.13	1.35	17.21	15.84	48.24	51.85	-3.61
12. $N_3P_3K_3$	10.74	18.33	1.94	20.08	13.64	64.08	69.09	-5.00
Average	**7.37**	**17.78**	**1.30**	**17.75**	**16.94**	**47.68**	**48.32**	**-0.64**

[1)] N_0 = 0N P_0 = 0P K_0 = 0K
N_1 = 150 kg N/ha P_1 = 75 kg P_2O_5/ha K_1 = 75 kg K_2O/ha
N_2 = 300 kg N/ha P_2 = 150 kg P_2O_5/ha K_2 = 150 kg K_2O/ha
N_3 = 600 kg N/ha P_3 = 300 kg P_2O_5/ha K_3 = 300 kg K_2O/ha

[2)] Sum of 4-5 cuts

[3)] Prices: cassava fresh roots: 1.2 baht/kg at 30% starch with a 0.02 baht reduction per 1% starch reduction
cassava leaves: 24 baht/kg crude protein

[4)] Costs: urea (46% N) baht 450/50kg Other costs: see footnote Table 7
TSP (46% P_2O_5) 1,200/50kg
KCl (60% K_2O) 440/50 kg

Without fertilization, the production cost was 26,360 baht/ha. Application of fertilizers increased production cost up to 69,090 baht/ha. The net incomes were negative or very low except for treatments $N_2P_0K_2$, $N_2P_1K_2$, $N_3P_2K_2$, $N_2P_2K_1$ and $N_2P_2K_2$ which offered some economic benefit ranging from 2,770 to 8,190 baht/ha. Therefore, for optimum total productivity, it is recommended to apply about 450 kg N, 150 kg K_2O and 0-150 kg P_2O_5/ha.

The leaf protein contents were not significantly different among various fertilization treatments (**Table 9**). However, application of high levels of N in combination with no or intermediate levels of K ($N_3P_2K_2$ and $N_2P_2K_0$) tended to maximize the protein and N contents of the leaves. The K content was highest at the highest level of applied K ($N_2P_2K_3$, $N_3P_3K_3$). The P, Ca and Mg levels in the leaves were highest with no N applications ($N_0P_0K_0$, $N_0P_2K_2$).

Plant Spacing Experiment for Leaf Production

The plant population trials had three cassava varieties i.e. Rayong 72, CMR 41-60-24 and Rayong 5 in main plots and four plant spacings, i.e. 60 x 60 cm, 50 x 50 cm, 40 x 40 cm and 30 x 30 cm in subplots.

At Rayong the dry leaf and fresh root yields and root starch contents were higher in 2002/03 than in 2003/04. In 2002/03, leaf and protein yields were similar among the three varieties though CMR 41-60-24 had slightly higher dry leaf and protein yields but lower root yields. The root yields were highest with Rayong 72 followed by those of Rayong 5. Leaf and protein yields were highest at the highest plant populations with the closests spacings of 30 x 30 cm regardless of the variety. Root yields had the opposite trend. Highest root yields were obtained from the widest spacing of 60 x 60 cm. Plant populations and spacing treatments seemed to have no effect on the root starch contents.

In 2003/04, similar results were obtained. Leaf and protein yields were highest with 30 x 30 cm spacing while root yields were highest with the 60 x 60 cm spacing.

In Khon Kaen the yield trends and root starch contents were slightly different from those obtained in Rayong. In 2002/03 Rayong 72 produced the highest dry leaf and protein yields and had the highest fresh root yields and starch contents. The widest plant spacing of 60 x 60 cm resulted in higher leaf protein and root yields and starch contents. In 2003/04, dry leaf and root yields were considerably lower than in 2002/03. No apparent effects of varieties and plant spacing could be noted, except for root yield. Rayong 72 produced twice the root yield of the other two varieties. On average, the root yield was highest with the wider spacings and lowest at the narrowest spacing.

When averaged over varieties, locations and years, the dry leaf yield ranged from 7.86 to 10.90 t/ha (**Table 10**). Root yields ranged from 17.0 to 21.6 t/ha, the dry leaf yield was highest with the closest spacing of 30 x 30 cm. Root yield, root starch content and net income increased with wider plant spacing (**Table 10**). To maximize the root yield and the economic benefit a plant spacing of 60 x 60 cm is recommended.

Table 9. Average total dry leaf yield, protein yield and nutrient removal, and the average protein and nutrient contents of the harvested leaves in the NPK trial for leaf production conducted at Rayong and Khon Kaen FCRC during 2002/03 and 2003/04.

Treatments[1)]	Total dry leaf yield (t/ha)	Total protein in leaves (kg/ha)	Total N removal in leaves (kg/ha)	Total P removal in leaves (kg/ha)	Total K removal in leaves (kg/ha)	Total Ca removal in leaves (kg/ha)	Total Mg removal in leaves (kg/ha)	Average protein content of leaves (%)	Average N content of leaves (%)	Average P content of leaves (%)	Average K content of leaves (%)	Average Ca content of leaves (%)	Average Mg content of leaves (%)
1. $N_0P_0K_0$	3.37	599	95.86	14.20	38.27	42.76	15.07	17.94	2.87	0.41	1.09	1.26	0.45
2. $N_0P_2K_2$	3.89	663	106.11	17.02	57.24	50.27	16.93	17.18	2.75	0.42	1.42	1.29	0.43
3. $N_1P_2K_2$	5.65	965	154.51	19.67	69.76	69.04	23.94	17.17	2.75	0.35	1.21	1.22	0.42
4. $N_2P_2K_2$	8.17	1,418	226.92	26.69	100.26	96.00	32.29	17.77	2.84	0.33	1.21	1.17	0.39
5. $N_3P_2K_2$	10.16	1,897	303.59	30.29	120.67	116.64	38.27	18.99	3.04	0.30	1.18	1.16	0.37
6. $N_2P_0K_2$	7.66	1,347	215.49	23.71	96.99	91.24	29.70	17.81	2.85	0.30	1.26	1.18	0.38
7. $N_2P_1K_2$	7.94	1,323	211.76	23.68	95.35	93.25	31.11	16.97	2.71	0.30	1.18	1.16	0.39
8. $N_2P_3K_2$	8.45	1,469	234.53	28.04	105.25	100.23	32.40	17.62	2.81	0.34	1.23	1.19	0.38
9. $N_2P_2K_0$	6.60	1,216	194.49	24.71	57.17	78.31	28.82	18.86	3.02	0.38	0.82	1.18	0.44
10. $N_2P_2K_1$	7.86	1,350	216.09	26.27	84.52	92.07	32.09	17.58	2.81	0.34	1.04	1.18	0.40
11. $N_2P_2K_3$	7.97	1,351	216.11	25.07	121.69	94.64	30.08	17.13	2.74	0.32	1.53	1.18	0.37
12. $N_3P_3K_3$	10.74	1,941	311.37	33.14	164.82	123.20	34.88	18.33	2.94	0.31	1.52	1.15	0.32
Average	**7.37**	**1,295**	**207.24**	**24.37**	**92.67**	**87.30**	**28.80**	**17.78**	**2.85**	**0.34**	**1.22**	**1.19**	**0.40**

1) N_0 = 0N; P_0 = 0P; K_0 = 0K
N_1 = 150 kg N/ha; P_1 = 75 kg P_2O_5/ha; K_1 = 75 kg K_2O/ha
N_2 = 300 kg N/ha; P_2 = 150 kg P_2O_5/ha; K_2 = 150 kg K_2O/ha
N_3 = 600 kg N/ha; P_3 = 300 kg P_2O_5/ha; K_3 = 300 kg K_2O/ha

Table 10. Average results of the plant population trials conducted at Rayong and Khon Kaen FCRC during 2002/03 and 2003/04.

Treatments[1)]	Total dry leaf yield[2)] (t/ha)	Average protein content (%)	Total protein yield[2)] (t/ha)	Fresh root yield (t/ha)	Root starch content (%)	Gross income[3)]	Production costs[3)]	Net income
						———(‘000 baht/ha)———		
V_1 -1	8.32	19.42	1.62	27.19	16.15	63.85	43.96	19.90
-2	8.02	19.03	1.53	26.96	16.61	61.73	45.24	16.49
-3	8.75	19.42	1.70	25.83	17.18	65.17	48.89	16.27
-4	10.87	18.14	1.97	23.76	16.63	69.50	57.44	12.06
V_2 -1	9.83	20.18	1.98	15.91	17.11	62.57	43.70	18.87
-2	7.98	19.71	1.57	13.85	17.43	50.88	42.97	7.91
-3	8.80	19.02	1.67	14.75	16.15	53.76	47.06	6.70
-4	11.01	19.07	2.10	10.06	15.68	59.59	55.26	4.33
V_3-1	8.06	21.40	1.73	21.71	17.78	62.14	42.75	19.40
-2	7.59	21.27	1.62	18.81	16.73	56.34	43.39	12.95
-3	8.71	19.45	1.70	18.85	17.34	58.53	47.66	10.86
-4	10.81	18.43	1.99	17.08	15.43	63.34	56.23	7.10
Average	**9.06**	**19.54**	**1.76**	**19.56**	**16.68**	**60.62**	**47.88**	**12.74**

Plant spacing	Total dry leaf yield (t/ha)				Average protein content (%)				Total protein yield (t/ha)			
(cm)	V_1[1)]	V_2	V_3	**Av.**	V_1[1)]	V_2	V_3	**Av.**	V_1[1)]	V_2	V_3	**Av.**
60 x 60	8.32	9.83	8.06	**8.74**	19.42	20.18	21.40	**20.33**	1.62	1.98	1.73	**1.77**
50 x 50	8.02	7.98	7.59	**7.86**	19.03	19.71	21.27	**20.00**	1.53	1.57	1.62	**1.57**
40 x 40	8.75	8.80	8.71	**8.75**	19.42	19.02	19.45	**19.30**	1.70	1.67	1.70	**1.69**
30 x 30	10.87	11.01	10.81	**10.90**	18.14	19.07	18.43	**18.55**	1.97	2.10	1.99	**2.02**
Average	**8.99**	**9.40**	**8.79**	**9.06**	**19.00**	**19.49**	**20.14**	**19.54**	**1.70**	**1.83**	**1.76**	**1.76**

Plant spacing	Fresh root yield (t/ha)				Root starch content (%)				Net income (‘000 B/ha)			
(cm)	V_1[1)]	V_2	V_3	**Av.**	V_1[1)]	V_2	V_3	**Av.**	V_1[1)]	V_2	V_3	**Av.**
60 x 60	27.19	15.91	21.71	**21.60**	16.15	17.11	17.78	**17.01**	19.90	18.87	19.40	**19.39**
50 x 50	26.96	13.85	18.81	**19.87**	16.61	17.43	16.73	**16.92**	16.49	7.91	12.95	**12.45**
40 x 40	25.83	14.75	18.85	**19.81**	17.18	16.15	17.34	**16.89**	16.27	6.70	10.86	**11.28**
30 x 30	23.76	10.06	17.08	**16.97**	16.63	15.68	15.43	**15.91**	12.06	4.33	7.10	**7.83**
Average	**25.93**	**13.64**	**19.11**	**19.56**	**16.64**	**16.59**	**16.82**	**16.68**	**16.18**	**9.45**	**12.58**	**12.74**

[1)] Varieties
V_1 = Rayong 72
V_2 = CMR 41-60-24
V_3 = Rayong 5

Plant spacing
1 = 60x60 cm = 27,778 plants/ha
2 = 50x50 cm = 40,000 plants/ha
3 = 40x40 cm = 62,500 plants/ha
4 = 30x30 cm = 111,111 plants/ha

[2)] Sum of 4-5 cuts

[3)] Prices and costs see footnote Table 7.

Cutting Height and Frequency Experiment for Leaf Production

For the cutting height and frequency experiment, treatments consisted of three cutting heights of 15, 20 and 25 cm in main plots and 4 cutting frequencies, i.e. cutting at 1½, 2, 2½ and 3 month intervals after the first cut at 2½ months after planting, in subplots. The variety was Rayong 72.

At Rayong the fresh leaf, dry leaf and root yields were higher in 2002/03 than in 2003/04. The root starch content was also higher in 2002/03 than in 2003/04. Fresh and dry leaf yields were highest at the low cutting height of 15 cm, but root yield was not affected. The lower cutting height reduced the root starch content especially in 2003/04. Cutting at 2½ month intervals increased the fresh and dry leaf yields; however, root yields and starch contents were highest when leaves were cut less frequently, i.e. at 3 month intervals in 2002/03 and at 2½ month intervals in 2003/04. Fresh and dry leaf yields decreased at cutting intervals of more than 2½ months.

Similar trends were found in Khon Kaen. At Khon Kaen, leaf and root yields were also higher in 2002/03 than in 2003/04. But the root starch contents were higher in 2003/04 than in 2002/03. This may be due to the lower amount of rainfall in 2003/04. In both years, leaf yields and root starch contents were not affected by varying the cutting heights from 15 to 25 cm, but the root yields were markedly higher at the greater cutting height of 25 cm in 2002/03.

Leaf cutting at greater intervals increased the fresh leaf, dry leaf and root yields regardless of the cutting heights, especially in 2002/03. However, fresh and dry leaf yields tended to decrease at cutting intervals greater than 2½ months in 2003/04. Less frequent cutting tended to increase the root starch content in 2003/04.

Table 11 shows that averaged over locations and years, the cutting interval of 2½ months resulted in the highest fresh and dry leaf yields, while the cutting at 3 month intervals maximized the fresh root yield and the root starch content. Fresh and dry leaf yields were highest at the lowest cutting height of 15 cm. In contrast, average fresh root yield and starch content were highest at the cutting height of 25 cm.

Net income varied between 2,870 to 18,490 baht/ha; the 2½ month cutting interval resulted in the highest net income.

From the results obtained in Rayong and Khon Kaen it may be concluded that varieties producing both high leaf and root yields include KU 50, Rayong 90, Rayong 72 as well as lines CMR 41-111-129 and CMR 41-42-3. These varieties or lines also produced the highest net income. To optimize root and leaf yields and maintain soil fertility it is recommended to apply a total of about 450 kg N, 75-150 kg P_2O_5 and 75-150 kg K_2O/ha. The optimum spacing is 60 x 60 cm while plants should be cut at 15-20 cm above the ground at 2½-3 month intervals. According to these management practices, cassava can produce 15-20 t/ha of roots yearly in addition to 10-13 t/ha of dry leaves containing 1.5-2.0 t/ha of crude protein; the latter is 2-3 times higher than a good crop of soybean.

Table 11. Average results of cassava cutting height x frequency trials for leaf production conducted at Rayong and Khon Kaen FCRC in Thailand during 2002/03 and 2003/04.

Treatments[1)]	Total dry leaf yield[2)] (t/ha)	Average protein content (%)	Total protein yield[2)] (t/ha)	Fresh root yield (t/ha)	Root starch content (%)	Gross income[3)]	Production costs[3)]	Net income
						———(‘000 baht/ha)———		
A -1	9.17	19.19	1.76	14.83	13.75	55.21	54.05	1.17
-2	10.48	16.59	1.74	20.89	14.44	60.26	56.20	4.07
-3	12.68	17.29	2.19	24.72	14.22	74.48	53.44	21.04
-4	10.81	16.10	1.74	28.22	16.53	68.02	51.66	16.36
B -1	9.00	19.86	1.79	17.28	14.50	58.28	54.28	4.00
-2	9.54	18.17	1.73	18.56	15.07	58.31	54.77	3.54
-3	11.01	17.76	1.96	22.98	15.20	67.69	51.30	16.39
-4	10.92	17.21	1.88	26.63	16.24	69.75	51.52	18.23
C -1	9.02	19.43	1.75	17.23	15.48	57.73	54.29	3.45
-2	9.96	18.16	1.81	22.18	15.66	63.64	55.85	7.79
-3	10.89	17.61	1.92	25.36	16.48	69.59	51.58	18.02
-4	10.03	16.83	1.69	28.19	16.80	66.89	50.80	16.09
Average	**10.29**	**17.85**	**1.83**	**22.26**	**15.36**	**64.15**	**53.31**	**10.85**

Cutting intervals (months)	Total dry leaf yield (t/ha) — Cutting height				Average protein content (%) — Cutting height				Total protein yield (t/ha) — Cutting height			
	15	20	25	**Av.**	15	20	25	**Av.**	15	20	25	**Av.**
1.5	9.17	9.00	9.02	**9.06**	19.19	19.86	19.43	**19.50**	1.76	1.79	1.75	**1.77**
2.0	10.48	9.54	9.96	**9.99**	16.59	18.17	18.16	**17.64**	1.74	1.73	1.81	**1.76**
2.5	12.68	11.01	10.89	**11.52**	17.29	17.76	17.61	**17.56**	2.19	1.96	1.92	**2.02**
3.0	10.81	10.92	10.03	**10.59**	16.10	17.21	16.83	**16.71**	1.74	1.88	1.69	**1.77**
Average	**10.78**	**10.12**	**9.97**	**10.29**	**17.29**	**18.25**	**18.01**	**17.85**	**1.86**	**1.84**	**1.79**	**1.83**

Cutting intervals (months)	Fresh root yield (t/ha) — Cutting height				Root starch content (%) — Cutting height				Net income (‘000 B/ha) — Cutting height			
	15	20	25	**Av.**	15	20	25	**Av.**	15	20	25	**Av.**
1.5	14.83	17.28	17.23	**16.45**	13.75	14.50	15.48	**14.58**	1.17	4.00	3.45	**2.87**
2.0	20.89	18.56	22.18	**20.54**	14.44	15.07	15.66	**15.06**	4.07	3.54	7.79	**5.13**
2.5	24.72	22.98	25.36	**24.35**	14.22	15.20	16.48	**15.30**	21.04	16.39	18.02	**18.49**
3.0	28.22	26.63	28.19	**27.68**	16.53	16.24	16.80	**16.52**	16.36	18.23	16.09	**16.89**
Average	**22.16**	**21.36**	**23.24**	**22.26**	**14.73**	**15.25**	**16.10**	**15.36**	**10.66**	**10.54**	**11.34**	**10.85**

Table 11. continued

[1] Cutting height
A = 15 cm above ground
B = 20 cm above ground
C = 25 cm above ground

Cutting frequency
1 = 1½ month intervals after 1 st cut
2 = 2 month intervals after 1 st cut
3 = 2½ month intervals after 1 st cut
4 = 3 month intervals after 1 st cut

[2] Sum of 4-5 cuts; [3] Prices and costs see footnote Table 7.

Several other experiments were conducted at the Thai Tapioca Development Institute (TTDI) Research and Training Center in Huay Bong, Thailand from 2002/03 to 2005/06. The station has limestone derived soils of fairly high pH (6.5-7.5), about 1% OM and with rather high levels of available P and exchangeable Ca, Mg and K. These experiments include several variety trials for leaf production, some of which have been reported by Watananonta *et al*., 2008. In one experiment ten varieties were planted at two planting distances, i.e. 30 x 30 cm and 60 x 60 cm. Total dry leaf yields and fresh root yields were highest at the wider spacing of 60 x 60 cm. Among the varieties tested, Huay Bong 60 produced the highest root yield and the second highest dry leaf yield, resulting in the highest gross and net incomes (**Table 12**).

Table 12. Total dry leaf and protein yield from five cuts of tops, final root yield and starch content as well as gross and net income obtained in a cassava variety x plant spacing trial for leaf production at TTDI Center in Huay Bong, Thailand in 2004/05. Data are for the 60 x 60 plant spacing.

Varieties	Total dry leaf yield (t/ha)	Average protein content (%)	Total leaf protein yield (t/ha)	Fresh root yield (t/ha)	Root starch content (%)	Gross income leaves ('000 baht/ha)	Gross income roots ('000 baht/ha)	Gross income total ('000 baht/ha)	Product. costs ('000 baht/ha)	Net income ('000 baht/ha)
Rayong 72	5.85	24.98	1.47	11.69	14.10	35.25	12.06	47.31	41.07	6.25
Rayong 90	5.85	24.10	1.42	11.34	16.83	33.99	12.33	46.32	40.99	5.33
KU 50	5.42	24.32	1.32	13.89	18.40	31.81	15.53	47.34	41.05	6.29
Huay Bong 60	8.34	24.71	2.08	15.16	14.40	49.80	15.74	65.54	44.86	20.68
CMR 35-22-196	4.61	25.94	1.21	8.10	10.97	28.99	7.85	36.84	38.76	-1.91
CMR 41-42-3	4.81	25.72	1.25	9.72	10.17	30.03	9.27	39.30	39.36	-0.06
CMR 41-60-24	5.38	25.46	1.37	9.55	16.27	32.88	10.27	43.15	40.01	3.14
CMR 41-61-59	6.69	25.22	1.68	10.01	9.87	40.26	9.48	49.74	41.68	8.06
CMR 41-111-129	8.71	24.13	2.08	12.04	11.63	49.80	11.83	61.63	44.58	17.05
CMR 41-114-125	6.16	25.45	1.55	9.38	9.90	37.13	8.89	46.02	40.90	5.12
Average	**6.18**	**25.00**	**1.54**	**11.09**	**13.25**	**36.99**	**11.32**	**48.32**	**41.32**	**6.99**

[1] Prices: cassava dry leaves: baht 24/kg protein
cassava fresh roots: 1.35/kg fresh roots at 30% starch, 0.02 baht reduction per 1% starch reduction

[2] Costs:

land preparation	baht 360/rai	fertilizer application	baht 400/rai
stakes	400/rai	weeding	600/rai
planting	250/rai	harvest, chopping, drying leaves	1,200/t dry leaves
fertilizers	3,007/rai	harvest + transport roots	230/t fresh roots

Note: 1 ha = 6.25 rai; 1 US$ = 40 baht in 2003/04

Another experiment on the effect of different levels and combinations of N, P and K for cassava leaf production, conducted with two varieties over two years, showed no significant effect of any NPK application on either total dry leaf or fresh root yields, even during the second year of planting. This indicates that in these very fertile soils there may not be a need for fertilizer inputs for several years, even with the frequent cutting of cassava tops for animal feeding.

Another experiment looked at the effect of plant spacing and row arrangements to maximize dry leaf and fresh root production, using two varieties, KU 50 and Rayong 72. **Table 13** shows the average results of this experiment. Highest leaf yields were obtained by the closest spacing of 30 x 30 cm followed by six rows planted at 30x30 cm and alternated by two empty rows for the tractor wheels. Highest fresh root yields, however, were obtained with the wider spacing of 60 x 60 cm. This also produced by far the highest net income.

Table 13. Total dry leaf and protein yields from five cuts of tops, final root yield and starch content as well as gross and net income obtained in a cassava plant spacing x variety trial for leaf production conducted at TTDI Center, Huay Bong, Thailand in 2004/05. Data are average values for two varieties, KU 50 and Rayong 72.

Spacing (cm)	Total dry leaf yield (t/ha)	Average protein content (%)	Total leaf protein yield (t/ha)	Fresh root yield (t/ha)	Root starch content (%)	Gross income [1] leaves ('000 baht/ha)	Gross income [1] roots ('000 baht/ha)	Gross income [1] total ('000 baht/ha)	Product. costs ('000 baht/ha)	Net income ('000 baht/ha)
30 x 30	4.23	24.37	1.03	24.40	17.20	24.75	26.64	51.39	50.48	0.91
45 x 45	3.03	24.49	0.74	22.12	17.48	17.81	24.37	42.18	42.21	-0.04
60 x 60	2.56	24.35	0.62	29.67	18.61	14.92	33.28	48.20	41.25	6.95
30 x 60	3.28	24.15	0.80	25.97	18.96	19.13	29.34	48.48	43.95	4.53
4(30 x 30) + 90	3.22	24.16	0.78	24.28	18.01	18.78	26.93	45.71	45.22	0.49
6(30 x 30) + 90	3.60	24.44	0.88	23.98	18.78	21.18	26.96	48.14	46.75	1.38
Average	**3.32**	**24.33**	**0.81**	**25.07**	**18.17**	**19.43**	**27.92**	**47.35**	**44.98**	**2.37**

[1] Prices: cassava dry leaves: baht 24/kg protein
cassava fresh roots: 1.35/kg fresh roots at 30% starch, 0.02 baht reduction per 1% starch reduction

Still another experiment studied the effect of cutting height and frequency as well as plant spacing for maximum leaf and root production using the variety KU 50. The results indicate that highest leaf yields were obtained with a cutting height of 20 cm, with cuttings at 1½ month intervals and at a spacing of 60 x 30 cm. However, highest root yields were obtained at a cutting height of 30 cm, a cutting frequency of every 2 months and a spacing of 60 x 60 cm. Combining the yields of dry leaves and fresh roots, the highest net income was obtained with a cutting height of 25 cm, a top cutting frequency of every 1½ months and a plant spacing of 60 x 30 cm. Naturally, the gross and net incomes depend on the relative prices of dry cassava leaves and fresh roots, which can change from year to year.

One other experiment was conducted on the effect of sprinkler irrigation and cutting frequency on leaf and root production. **Table 14** shows that irrigating cassava monthly increased both leaf and root production as well as the protein yield, but bimonthly irrigation produced a slightly higher protein content of the leaves and starch content of the roots, resulting in the highest net income. Concerning cutting frequency, cutting at 2½ month intervals produced the highest leaf yields, but cutting at 3 month intervals produced the highest root yields.

Table 14. Effect of irrigation and cutting frequency on the dry leaf and fresh root yield as well as the gross and net income obtained during a one year cropping cycle of cassava, cv. KU 50, at TTDI Center in Huay Bong, Thailand in 2005/06.

Treatments [2]	Dry leaf yield (t/ha)	Leaf protein content (%)	Leaf protein yield (t/ha)	Fresh root yield (t/ha)	Root starch content (%)	Gross income [1] roots ('000 baht/rai)	Gross income [1] leaves ('000 baht/rai)	Product. costs ('000 baht/rai)	Net income ('000 baht/rai)
A-T1	9.90	26.36	2.21	16.10	20.87	16.52	69.32	50.47	35.38
-T2	9.30	24.82	2.29	14.56	22.00	15.43	65.10	51.11	29.42
-T3	10.77	24.64	2.62	15.08	20.25	15.19	75.41	54.64	35.95
-T4	8.71	24.26	2.12	22.02	20.55	22.39	60.97	50.06	33.29
B-T1	10.74	26.51	2.75	17.86	21.05	18.42	75.21	59.33	34.30
-T2	10.96	26.08	2.81	16.75	21.90	17.71	76.69	59.84	34.55
-T3	10.15	25.10	2.50	19.75	20.90	20.28	71.03	58.05	33.26
-T3	9.95	24.28	2.41	21.61	20.20	21.74	69.67	57.44	33.97
C-T1	11.82	26.60	3.10	19.62	20.60	19.97	82.70	66.42	36.26
-T2	9.87	25.78	2.56	16.36	20.27	16.50	69.10	60.36	25.24
-T3	12.14	24.90	3.01	23.83	19.12	23.20	85.01	68.64	39.56
-T4	9.32	24.21	2.24	24.35	18.05	22.92	65.20	60.44	27.68
Average	**10.30**	**25.29**	**2.55**	**18.99**	**20.48**	**19.19**	**72.12**	**58.07**	**33.24**

[1] Prices: cassava dry leaves: baht 7000/ton
cassava fresh roots: baht 1.30/kg at 30% starch with 0.03 baht reduction per 1% starch reduction

[2] T-1 = 1st cut at 2½ months; subsequent cuts at 1½ month intervals; total 6 cuts
T-2 = 1st cut at 2½ months; subsequent cuts at 2 month intervals; total 6 cuts
T-3 = 1st cut at 2½ months; subsequent cuts at 2½ month intervals; total 5 cuts
T-4 = 1st cut at 2½ months; subsequent cuts at 3 month intervals; total 4 cuts

A = no irrigation
B = 25 mm applied every two months if rainfall < 100 mm in previous month
C = 25 mm applied every month if rainfall < 100 mm in previous month

Table 14. continued

Cutting intervals (months)	Total dry leaf yield (t/ha)				Average protein content (%)				Total protein yield (t/ha)			
	Irrigation				Irrigation				Irrigation			
	A	B	C	**Av.**	A	B	C	**Av.**	A	B	C	**Av.**
1.5	9.90	10.74	11.82	**10.82**	26.36	26.51	26.60	**26.49**	2.21	2.75	3.10	**2.68**
2.0	9.30	10.96	9.87	**10.04**	24.82	26.08	25.78	**25.56**	2.29	2.81	2.56	**2.55**
2.5	10.77	10.15	12.14	**11.02**	24.64	25.10	24.90	**24.88**	2.62	2.50	3.01	**2.71**
3.0	8.71	9.95	9.32	**9.33**	24.26	24.28	24.21	**24.25**	2.12	2.41	2.24	**2.26**
Average	**9.67**	**10.45**	**10.78**	**10.30**	**25.02**	**25.49**	**25.37**	**25.29**	**2.31**	**2.62**	**2.73**	**2.55**

Cutting intervals (months)	Fresh root yield (t/ha)				Starch content (%)				Net income ('000 baht/rai)			
	Irrigation				Irrigation				Irrigation			
	A	B	C	**Av.**	A	B	C	**Av.**	A	B	C	**Av.**
1.5	16.10	17.86	19.62	**17.86**	20.87	21.05	20.60	**20.84**	35.38	34.30	36.26	**35.31**
2.0	14.56	16.75	16.36	**15.89**	22.00	21.90	20.27	**21.39**	29.42	34.55	25.24	**29.74**
2.5	15.08	19.75	23.83	**19.55**	20.25	20.90	19.12	**20.09**	35.95	33.26	39.56	**36.26**
3.0	22.02	21.61	24.35	**22.66**	20.55	20.20	18.05	**19.60**	33.29	33.97	27.68	**31.65**
Average	**16.94**	**18.99**	**21.04**	**18.99**	**20.92**	**21.01**	**19.51**	**20.48**	**33.51**	**34.02**	**32.19**	**33.24**

The final experiment looked at the economics of cutting plant tops, either once at final root harvest at 11½ MAP or from 1 to 5 cuts during the growth cycle. These cuts were made at 2½, 5, 7, 8 and 11½ MAP (**Table 15**).

Table 15. Average effect of the number and timing of leaf cutting on the total dry leaf and protein yields, root yield and starch content of two cassava varieties as well as gross and net income obtained in an experiment at TTDI Center in Huay Bong, Thailand.

Leaf cut no.					Total dry leaf yield	Average protein content	Total leaf protein yield	Fresh root yield	Root starch content	Gross income[1)]			Prod. costs	Net income
1	2	3	4	5	(t/ha)	(%)	(t/ha)	((t/ha)	(%)	leaves	roots	total ('000 B/ha)		
				x	0.71	24.46	0.17	39.89	19.58	4.15	45.43	49.58	24.30	25.28
x				x	1.50	25.16	0.38	39.91	20.15	9.02	46.01	55.04	30.68	24.35
x	x			x	1.99	25.21	0.50	27.02	21.10	11.92	31.59	43.51	32.53	10.99
x	x	x		x	2.56	25.13	0.64	28.60	19.75	15.34	32.53	47.88	36.78	11.09
x	x	x	x	x	2.57	25.28	0.65	24.46	18.19	15.56	27.20	42.76	40.07	2.70
Average					**1.87**	**25.05**	**0.47**	**31.97**	**19.75**	**11.20**	**36.55**	**47.75**	**32.87**	**14.88**

[1)] Prices: cassava dry leaves: 24 B/kg protein
cassava fresh roots: 1.35 B/kg fresh roots at 30% starch, 0.02 baht reduction per 1% starch reduction

Table 15 shows that total dry leaf yields increased from one to five cuts, but that the root yields decreased with increasing number of cuts. Considering the prices of fresh roots and dry leaves at the time of the experiment, the highest gross income from the sale of both roots and dry leaves was obtained with only two cuts, at 2½ MAP and at the time of root harvest, while the highest net income was obtained with only the final cut at time of root harvest. Thus, under the price and cost scenarios of 2004/05 it was not economic to prune the tops several times during the growth cycle for sale of dry leaves, as this had a negative effect on root production and net income.

Similar results were obtained from two experiments conducted at Jatikerto Experiment Station in Malang, Indonesia, as reported by Utomo *et al.,* 2010. In the first experiment they planted three cassava varieties at either 100 x 80 cm or 100 x 40 cm with intercropped maize planted along the cassava rows. Cassava plants were either not pruned (but the young shoots harvested at time of root harvest), whole tops cut at 30 cm above the ground at 3 MAP and at 2 month intervals thereafter, or only the leaves removed following the same schedule as the top pruning. Intercropped maize yields were not affected by the various cassava pruning regimes, but root yields were seriously reduced, especially when the whole tops were removed (Utomo *et al.,* 2010).

In the second experiment, cassava, UB 477-2, was planted and pruning was done by cutting off the whole tops at about 30 cm above the ground; the first pruning was done at about 2½ months, after which tops were pruned at 2 month intervals. The results, given in **Table 16**, show that cutting off the cassava tops four times during the growth cycle significantly decreased the root yields, at both plant spacings. Application of N fertilizer to the pruned cassava, up to 600 kg urea/ha, increased both the leaf and root yields. However, the intercropped maize yields were not significantly influenced by the cassava pruning or spacing treatments, nor by the rate of N application. It seems that the application of 300 kg urea/ha was sufficient to meet the maize requirement in the pruned cassava system.

Furthermore, data in **Table 16** shows that under the current prices and experimental conditions the net income from growing cassava for both leaf and root production, using a cassava + maize intercropping system, was less profitable than growing cassava without leaf pruning, and that high rates of N application are required to increase the net income when tops are cut off regularly for leaf production.

Table 16. Effect of leaf pruning, plant spacing and rate of N application on the leaf and root yields of cassava and the yield of intercropped maize, as well as on the gross and net income when cassava, UB 477-2, was grown in Jatikerto, Malang, Indonesia, in 2005/06.

Pruning/spacing/urea rate	Maize yield (t/ha)	Dry leaf yield (t/ha)	Fresh root yield (t/ha)	Gross income maize grain[2)]	Gross income cassava leaves[2)]	Gross income cassava roots[2)]	Pro-duction costs[3)]	Net income
				('000 Rp/ha				
No pruning/1.0x0.8/300	3.27	1.34[1)]	46.76	5,232	2,010	18,704	5,237	20,709
No pruning/1.0x0.4/300	2.64	1.68[1)]	48.68	4,224	2,520	19,472	5,516	20,700
Leaf pruning/1.0x0.8/300	3.68	3.14	10.11	5,888	4,710	4,044	5,782	8,860
Leaf pruning/1.0x0.8/400	3.97	4.32	13.62	6,352	6,480	5,448	6,820	11,460
Leaf pruning/1.0x0.8/500	4.04	6.27	15.82	6,464	9,405	6,328	8,378	13,819
Leaf pruning/1.0x0.8/600	3.97	7.73	18.09	6,352	11,595	7,236	9,590	15,893
Leaf pruning/1.0x0.4/300	2.97	4.18	12.27	4,752	6,270	4,908	6,563	9,367
Leaf pruning/1.0x0.4/400	3.17	5.19	15.81	5,072	7,785	6,324	7,481	11,700
Leaf pruning/1.0x0.4/500	3.05	6.32	19.02	4,880	9,480	7,608	8,478	13,490
Leaf pruning/1.0x0.4/600	3.43	9.07	20.57	5,488	13,605	8,228	10,591	16,730

[1)] Cassava leaves at time of root harvest only

[2)] Prices: maize Rp 1,600/kg dry grain
cassava roots 400/kg fresh roots
cassava leaves 1,500/kg dry leaves

[3)] Costs: (Rp)

	Cassava monoculture	Cassava+maize
land preparation (40 md/ha)	700,000/ha	700,000/ha
planting	225,000/ha	285,000/ha
weeding+hilling up (21 md/ha)	375,000/ha	375,000/ha
fertilizer+manure application	180,000/ha	270,000/ha
harvesting+loading cassava	17,000/t fresh roots	20,000/t fresh roots
harvesting maize	-	75,000/ha
maize seed	-	250,000/ha
fertilizers -urea (1,300/kg)	390,000 (300 kg/ha)	520,000 (400 kg/ha)
-SP 36 (1,600/kg)	160,000 (100 kg/ha)	160,000 (100 kg/ha)
-KCl (3,000/kg)	345,000 (115 kg/ha)	345,000 (115 kg/ha)
-manure (100/kg)	500,000 (5 t/ha)	500,000 (5 t/ha)
leaf harvesting+transport	300,000/t dry leaves	300,000/t dry leaves
leaf chopping + drying	410,000/t dry leaves	410,000/t dry leaves

***Source**: Utomo et al., 2010.*

SUMMARY AND CONCLUSIONS

Based on these experiments the following conclusions can be drawn:

1. The green top growth of cassava can be cut regularly to produce foliage that is high in protein and suitable for direct feeding to animals, either after wilting, drying or ensiling; or it can be used as feedstock for production of commercial animal feed.
2. After cutting off the tops the remaining stem will resprout again to produce more foliage, which can be cut again, resulting in up to 4-5 cuts in a one-year crop cycle. The crude protein content of this forage varies from about 18 to 25%.

3. Usually, the first cut is made at 2½ months after planting, and subsequent cuts at 1½, 2 or 3 month intervals, or according to the growth of the plants, which is markedly reduced during the dry season. Cutting tops at short intervals tend to result in high foliage yields but reduced root yields. When cassava tops were cut three times during a one year crop cycle, root yields were reduced 35-50%; when tops were cut six times during a one year crop cycle in Indonesia root yields were reduced nearly 75%, making the leaf production system uneconomic.
4. There are large varietal differences in their suitability for leaf production. Experiences in Colombia indicate that varieties well-adapted to cool climates at high elevations will produce very vigorous top growth when planted at lower elevations or in warmer climates. However, under these conditions these varieties tend to have rather low root yields. If the objective is to produce both high yields of leaves without sacrificing too much root yield, it may be better to plant those varieties that are known to produce high root yields and then stimulate their top production with high applications of N.
5. The need for fertilization is obviously dependent on the native fertility of the soil. In some very fertile soils, like those at TTDI in Thailand, the crop may not need any fertilizers for several years. Soils very low in K, as those in Quilichao, Colombia, will need rather high applications of both N and K, while the low-N soils of Jatikerto will mainly need high applications of N. In general, however, the high protein content of cassava leaves also mean that high leaf yields will remove large amounts of N (up to 700 kg N, **Table 6**) and this is the nutrient that is required in largest quantities for high production of leaves, while high levels of K are required to maintain high yields of roots.
6. The height of cutting may not be that important for increasing yields, but does affect the balance between root and leaf yields. Cutting close to the ground will increase leaf yields but also decrease root yields, while cutting higher up the stem will do the opposite. Thus, depending on the relative prices of fresh roots and dry leaves, one has to determine the right balance between root and leaf production for maximizing net income.
7. Applying irrigation during long dry periods will tend to increase the yields of both roots and leaves, but may also decrease the protein content of leaves and the starch content of roots. It will also increase considerably the cost of production and thus may or may not be economically justified.
8. The best plant spacing tends to be somewhere between 30 x 30 cm and 60 x 60 cm, the closer spacing favoring high leaf production and the wider spacing favoring root production.
9. In many cases the frequent cutting of cassava tops will sacrifice root yields to such an extent that it becomes uneconomic to produce these leaves. This, of course depends on the relative prices of fresh roots and dry leaves, and on the yield levels obtained. To become more economic the cost of high density planting and the harvesting of cassava leaves will need to be reduced, mainly by mechanizing these operations, as well as the transport, chopping and drying of the cassava tops, as these operations are still very labor intensive. Moreover, production costs can be reduced by harvesting the roots only after two or more years, which allows for at least 7-8 cuts of foliage during a two-year crop cycle. This will markedly increase both the root and foliage yields.

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CHAPTER 25

FRESH AND ENSILED CASSAVA ROOTS AND FOLIAGE FOR SWINE AND RUMINANTS [1]

Julián Buitrago A., DVM, PhD. [2]

INTRODUCTION

The usual feeding practice in most traditional experiences with fresh cassava has been the daily supply of the whole chopped roots supplemented with a dry mixture of protein and micro ingredients (vitamins, minerals and feed additives). As anticipated, this practice is mainly suitable for small or medium size swine and cattle enterprises where cassava production is usually a complement to the animal operations and where hand labor is not an important limitation.

For larger and more technified operations, the heavy hand labor requirements, the perishability of the product and the troublesome management of the daily feeding program, limit the extensive use of fresh products. The use of dried mixtures in automatic feeding systems is the general trend in these cases, where cassava roots and/or foliage should be dried and, preferably, pelletized, to be included in commercial diets.

Although the information with fresh and ensiled roots for swine and cattle feeding is quite lengthy, a summarized report of the most relevant studies is included, with special emphasis on the experimental work conducted at CIAT.

PERFORMANCE RESULTS WITH FRESH CASSAVA ROOTS IN SWINE FEEDING

Programs based on fresh cassava are suitable for feeding growing-finishing pigs and breeding sows. Due to the high moisture and low energy of roots, the animals have to be supplied with ample amounts of chopped cassava roots and a limited amount of a dry protein supplement. Nevertheless, in most cases the animal is not able to consume the total energy requirements even though fresh cassava is offered at free choice. The maximum consumption of fresh roots obtained in most studies is around 3 kg for growing pigs, 4 kg for finishing pigs and 6 kg for lactating gilts, which is less than the expected consumption of 3.5-4, 5-6 and 8-10 kg, respectively. Based on these limitations, the performance is partially affected although in several cases the cost:benefit criteria is positive for the small producer.

In the day-to-day feeding management program, cassava can either be supplied in a mixture together with the nutritional supplement or separately. Nevertheless, free choice supply of the supplement often results in over-consumption of protein, minerals and vitamins, which generally raises the price and makes the feeding program inefficient.

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[1] For color photos see pages 786-789.

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The most recommended programs to fulfill the pigs' nutritional requirements and at minimal production costs are based on nutritional supplement supply in a daily controlled scheme, according to age and weight of the animals.

Fresh cassava from sweet varieties can be supplied either at free choice to pigs or in controlled amounts to avoid waste, although consumption should not be restricted. Each day, the corresponding amount of fresh roots must be offered to the animals.

When pigs weigh less than 50 kg, they consume smaller amounts of fresh cassava (2.5-3.5 kg/day), but afterwards, during the final fattening stages, consumption should increase up to 4.0-4.5 kg/day. Since these quantities still do not provide the pig with the required dry matter (DM) or energy level to obtain maximum performance, the animal tries to compensate this deficit with a higher consumption of the nutritional supplement (in the case that it is offered at free choice).

In the following tables, results of different trials with growing-finishing pigs and breeding females are analyzed.

Fresh Cassava Roots for Growing-Finishing Pigs

Tables 1 to **6** illustrate different feeding approaches which have been tested as viable alternatives to maximize the consumption of fresh roots and to avoid the over consumption of protein supplement without affecting the performance of animals.

Table 1. The effect of using fresh cassava roots and protein supplements in free choice supply to Duroc x Landrace growing pigs (15-50 kg) [1] on their performance.

Supplement ingredients %	**1**	**2**	**3**	**4**
Cottonseed meal	16.0	23.0	23.0	--
Sesame meal	18.0	25.0	--	25.0
Peanut meal	14.0	--	25.0	23.0
Fish meal	36.0	36.0	36.0	36.0
Meat meal	14.2	14.2	14.2	14.2
Lysine	0.2	0.2	0.2	0.2
Vitamin premix	0.6	0.6	0.6	0.6
Nutrient composition				
Digestible energy, Mcal/kg	2.85	2.83	2.88	2.77
Protein, %	54.2	53.9	56.0	52.9
Methionine, %	1.20	1.27	1.07	1.23
Lysine, %	3.19	3.18	3.28	3.15
Performance of pigs				
Daily weight gain, kg	0.59	0.57	0.64	0.53
Daily feed consumption:				
Fresh cassava, kg	3.24	3.24	3.15	2.98
Protein supplement, kg	0.50	0.45	0.52	0.51
Feed conversion ratio (DM)	2.66	2.63	2.44	2.79

[1] Chopped fresh cassava roots and protein supplement offered in different feeders for free-choice consumption.

Source: *Contreras, 1973.*

The information obtained from the performance results confirms most of the observations already mentioned and illustrates some new approaches to be considered for a more efficient use of fresh roots.

In general, performance results are a little lower than those obtained with commercial corn-soybean meal diets. The main reason is associated with a lower consumption of DM when cassava roots are fed fresh, due to the incapacity of the pig to consume larger levels of the fresh product. The high water content and probably the effect of low levels of HCN still present in sweet varieties of cassava roots may also have some influence in this situation.

When the protein supplement is provided in a free choice arrangement, the animals will consume larger amounts as compensation to the reduced consumption of cassava roots. Therefore, an over-consumption of protein (approximately an extra 20%) will occur, which results in the higher cost of the total diet (**Tables 2** and **4**).

Table 2. The effect of using fresh cassava roots and protein supplements in free choice vs. controlled supply for Duroc growing-finishing pigs (18-100kg)[1] on their performance.

Parameter	Free choice fresh roots + protein supplement[2]		Corn-SBM diet[3]
	Controlled supplement	Free choice supplement	
Soybean meal, %	61.50	61.50	10.59
Cottonseed meal, %	20.50	20.50	3.53
Minerals and vitamins, %	18.00	18.00	4.55
Corn, %	--	--	81.33
Daily consumption			
Fresh roots, kg	3.89	4.05	--
Protein supplement, kg	0.73	1.17	--
DM consumption, kg	2.07	2.52	2.60
Protein consumption, kg	0.372	0.564	0.459
Performance of pigs			
Daily weight gain, kg	0.79	0.83	0.84
Feed conversion ratio (DM)	2.90	3.36	3.43

[1] Chopped fresh cassava and protein supplement offered in different feeders in free-choice or controlled consumption

[2] Protein supplement with 43% protein

[3] Commercial concentrates with 16% protein

Source: *Buitrago, 1964.*

The over-consumption of protein supplement is observed regardless of the ingredients used in the formulation, but the inclusion of intermediate levels of meat meal and blood meal seem to stimulate a further increase in the daily consumption (**Table 4**).

As a mechanism to avoid the over-consumption of the protein supplement, it should be offered every day in controlled amounts related with the body weight of the pig. Although the protein consumption is controlled, the total consumption of DM is still

deficient due to the lower cassava intake, which partially affects the animal's performance (**Table 2**).

In **Table 3** it can be observed that the addition of sugarcane molasses or raw sugar to the cassava roots resulted in a small increase in consumption of roots and DM, and a lower consumption of the protein supplement, which improves the energy:protein ratio as well as the performance of the pigs.

Table 3. Fresh roots and protein supplement added with molasses or sugarcane for Yorkshire growing-finishing pigs (20-90 kg).

Parameter	Feeding regime[1)]		
	Only roots	Roots + Molasses	Roots + Sugar
Daily consumption (kg)			
Fresh cassava roots	2.99	3.27	3.11
Protein supplement (40% protein) [2)]	1.02	0.92	0.85
Total DM	2.03	2.27	2.17
Total protein	0.54	0.51	0.46
Pig performance			
Daily weight gain, kg	0.69	0.72	0.74
Feed conversion rate (DM)	2.97	3.16	2.93

[1)] Molasses and sugarcane were used in a proportion equivalent to 15% of the total diet.
[2)] Protein supplement based on soybean meal (80.0%), corn (8.5%) and minerals & vitamins (11.5%). Free choice supply in feeders separated from the cassava treatments.
***Source**: CIAT, 1975.*

Lowering the protein content of the supplements also helps in reducing protein consumption in pigs, although the consumption of fresh roots is also reduced. The total DM intake from cassava roots are reduced, while the supplement consumption and weight gains are improved by providing lower protein percentages, which also results in a better feed conversion (**Table 5**).

When bitter varieties (e.g. CMC-84) of fresh cassava roots are used, an additional decrease in its consumption is observed with a parallel increase in the consumption of protein supplement when it is offered *ad labium* (**Table 6**). However, when the protein supplement is controlled to the required daily level, both the cassava and the protein supplement consumption are reduced, creating a larger deficit in the daily DM (energy) intake and a drastic reduction in animal performance.

Fresh Cassava Roots for Gestating and Lactating Gilts

A small number of studies with fresh cassava roots have been conducted during gestation and lactation. While gestating females need small amounts of DM (energy) to fulfill their requirements, lactating females require 2 to 4 times more intakes of energy as well as protein. Therefore, the reduced consumption of cassava roots should not be an important limiting factor in gestation, in contrast to the high demand during lactation.

Table 4. Fresh roots and protein supplements prepared with different protein sources for Duroc x Landrace growing-finishing pigs (19-90 kg) [1)].

Parameters	Protein sources (%)					
Soybean meal	78.10	--	--	--	--	--
Cottonseed meal	--	--	78.10	--	30.00	30.00
Meat meal	--	70.50	--	44.30	21.30	--
Blood meal				20.00	20.00	--
Fish meal	--	--	--	--	--	36.70
Corn	11.20	26.80	11.20	33.00	25.00	29.60
Vitamins & minerals	10.70	2.70	10.70	2.70	10.70	10.70
Protein level (%)	**43.0**	**39.4**	**37.7**	**48.5**	**44.7**	**40.2**
Daily consumption (kg)						
Fresh roots	4.00	3.40	3.13	3.88	4.00	4.08
Protein supplement	0.80	0.78	0.79	0.94	0.90	0.79
Total Protein	0.34	0.31	0.30	0.44	0.40	0.32
Pig performance						
Daily weight gain, kg	0.72	0.68	0.59	0.72	0.72	0.68
Feed conversion rate	3.25	3.07	3.38	3.32	3.38	3.47

[1)] Both cassava roots and protein supplements were supplied at free choice in separated feeders.
***Source**: Maner et al., 1978.*

Table 5. Fresh roots and protein supplements with different protein levels for Yorkshire growing-finishing pigs (19-90 kg) .

Supplement ingredients (%)			
Soybean meal	26.73	53.15	79.56
Corn	67.27	37.85	8.44
Minerals and vitamins	6.0	9.0	12.0
Protein level (%)	**20.0**	**30.0**	**40.0**
Daily consumption (kg)			
Fresh roots	1.79	2.74	3.37
Protein supplement	1.39	1.00	0.75
Total DM	1.92	1.94	1.97
Total protein	0.34	0.40	0.39
Pig performance			
Daily weight gain, kg	0.71	0.67	0.65
Feed conversion rate	2.71	2.90	3.02

***Source**: CIAT, 1974.*

Table 7 summarizes the feed treatments and the performance results of gestating gilts kept on pasture or in confinement. Both cassava roots and the 40% protein supplement

were offered in controlled amounts to supply the daily requirements. The feeding of gilts on pasture was adjusted so they received smaller amounts of cassava and protein supplementation since the pasture provided part of the requirements.

Table 6. Performance of Yorkshire pigs fed with sweet vs. bitter cassava roots plus a protein supplement[1] with different protein levels.

	Sweet roots		Bitter roots[2]	
	Free choice supplement	Controlled supplement	Free choice supplement	Controlled supplement
Daily consumption (kg)				
Fresh roots	2.99	3.40	0.98	0.93
Protein supplement	0.81	0.82	1.21	0.22
Total DM	1.78	1.80	1.43	0.52
Pig performance				
Daily weight gain (kg)	0.66	0.77	0.56	-
Feed conversion ratio	2.99	2.61	2.86	-

[1] 40% protein supplement in all treatments
[2] CMC-84 variety with 200 ppm cianhydric acid
Source: *CIAT, 1973.*

Table 7. Fresh cassava roots and protein supplementation in Duroc x Landrace gestating gilts.

	Feed treatment		
	Control pasture[1]	Cassava + supplement pasture[2]	Cassava + supplement confined[3]
Ingredients (%)			
Soybean meal	18.0	64.08	66.75
Cottonseed meal	--	20.53	20.53
Corn	74.8	--	--
Minerals and vitamins	7.20	15.39	12.72
Protein level (%)	**16.0**	**40.0**	**40.0**
Performance of gilts			
Weight gain in gestation, kg	19.90	24.90	37.70
Piglets / litter, No	10.4	10.0	7.7
Piglet weight, kg	1.28	1.12	1.18
Litter weight, kg	13.31	11.20	9.08

[1] Daily consumption/gilt: 1 kg of a corn - soybean meal diet.
[2] Daily consumption/gilt: 1.7 kg of cassava roots and 0.4 kg of protein supplement.
[3] Daily consumption/gilt: 3.1 kg of cassava roots and 0.62 kg of protein supplement.
Source*: Maner et al., 1978.*

The daily feed intake of cassava and protein supplement corresponded to the predicted daily need of DM and protein during gestation. While cassava fed gilts gained

more weight during gestation, the litters were smaller and lighter. Piglet weight and litter weight at birth was lower in the cassava treatments, especially when gilts were confined.

On the other hand, the performance of sows and litters during lactation was not affected by the inclusion of cassava roots and protein supplement in a balanced proportion (**Table 8**). The mixture of cassava roots and protein supplement was equivalent to a 16% protein diet on a DM basis, which is similar to the control group given a corn-soybean meal diet.

Daily consumption of DM in the cassava group was smaller (3.40 kg) than in the control group (4.32 kg). In spite of the reduced consumption of DM, total litter weight at weaning was not affected, even with the smaller litter size of the cassava fed sows. The sows from the control group gained a little more weight during lactation since their DM consumption was higher.

Table 8. Fresh cassava roots and protein supplementation as compared to a corn-soybean meal ration in Duroc x Landrace lactating sows.

	Corn-SBM[1)]	Fresh roots + protein supplement[2)]
Ingredients (%)		
Soybean meal	15.00	87.10
Corn	81.35	--
Minerals and vitamins	3.65	12.90
Protein level (%)	**16.0**	**40.0**
Daily consumption (kg)		
Corn-soybean meal diet	4.82	--
Fresh cassava	--	6.50
Protein supplement	--	1.21
Total DM intake	4.32	3.40
Performance of sows		
Weight at farrowing, kg	179.30	158.30
Weight at weaning, kg	190.30	165.80
Performance of litter at birth		
No. piglets	10.8	9.3
Individual weight, kg	1.18	1.36
Litter weight, kg	12.74	12.65
Performance of litter at weaning (35 days)[3)]		
No. piglets	9.0	7.6
Individual weight, kg	6.03	7.63
Litter weight, kg	54.27	58.00

[1)] Control group with free choice consumption.
[2)] Cassava roots and protein supplement in a mixture to provide the equivalent to a 16% protein diet. Free choice consumption.
[3)] Piglets received the same feed at free choice; **Source**: *Maner et al., 1978.*

PERFORMANCE RESULTS WITH ENSILED CASSAVA ROOTS IN SWINE FEEDING

A large proportion of the information obtained with fresh cassava in animal feeding also applies to the preserved product obtained through the silage process. The principal nutritional differences are due to the starch fermentation and the reduction in moisture during the silage production process. Again, monogastric animals, like swine and poultry, generally are not able to consume the total amount of DM from the ensiled roots to satisfy the energy requirements during the higher demanding phases. Their performance is slightly affected in terms of weight gains, although feed efficiency and production costs will probably compensate for the slower weight gain. Growing-finishing pigs, gilts and sows are suitable to be included in feeding programs based on cassava silage, once the performance limitations are considered.

As was already mentioned with the fresh cassava feeding practices, the ensiled product also has to be offered in a day to day scheme. Protein supplementation can be offered at free choice or in daily controlled amounts. However, the most recommended feeding practice consists in *ad libitum* supply of ensiled chopped roots plus a controlled quantity of protein supplement which has to be periodically calculated to fix the precise amount to be offered.

Ensiled Cassava Roots for Growing-Finishing Pigs

The following information on the performance of pigs included in different feeding demonstrations with ensiled cassava, considers the use of ensiled cassava roots in a free choice supply and the controlled supply of protein supplement.

Table 9 refers to growing finishing pigs which were fed three possible cassava-based feeding schemes: fresh roots, ensiled roots, and ensiled roots plus foliage. In all cases, the cassava products were supplemented with a fixed amount of protein supplement (38% protein) to satisfy the daily requirements.

From the performance results it may be concluded that the silage process of cassava roots is a valid alternative to be considered as a mechanism to preserve their nutritional value. The high perishability of the fresh roots may be overcome through the inexpensive practice of anaerobic silage production, which also facilitates the feeding management practices for the small- and medium-size producer.

Table 9 shows a very similar response in weight gains and feed efficiency when fresh roots are compared with ensiled roots on a DM basis. However, the inclusion of cassava foliage to the ensiled product negatively affected the consumption of the silage, which is reflected in lower weight gains and poorer feed conversion ratios. The lower consumption of the combined roots and foliage silage may be related to the lower palatability of leaves and stems even at minimum levels (10%).

The information presented in **Table 10** illustrates the possibilities to include different ingredients as protein supplements to cassava silage in growing finishing pigs. Excluding the high fish meal supplement, where the consumption was reduced, these alternatives compare favorably with pigs fed commercial balanced diets.

Table 9. The effect of using fresh cassava roots compared with ensiled cassava roots and foliage for Yorkshire x Landrace growing-finishing pigs (18-98 kg).

	Ensiled roots[1)]	Ensiled roots+foliage[2)]	Fresh roots
Supplement ingredients (%)			
Corn	10.9	10.9	10.9
Cottonseed meal	78.1	78.1	78.1
Vitamins & minerals	11.0	11.0	11.0
Daily consumption (kg)			
Ensiled cassava roots (and foliage)	3.84	3.05	--
Fresh cassava roots	--	--	4.04
Protein supplement (38%)	1.01	1.01	1.01
Total protein	0.38	0.38	0.38
Pig performance			
Daily weight gain (kg)	0.77	0.64	0.75
Feed conversion ratio (DM)	2.92	3.17	3.09

[1)] Only chopped roots
[2)] Chopped roots, leaves and stems
Source: *Buitrago et al., 1978.*

Table 10. The effect of feeding ensiled cassava roots with different protein supplements to Yorkshire growing-finishing pigs (16-90 kg) on their performance.

	Ensiled roots plus protein supplement				Corn-SBM diet
Supplement ingredients (%)					
Soybean meal	44.0	--	88.0	--	8.5
Cottonseed meal	44.0	48.5	--	97.0	8.5
Fish meal	--	48.5	--	--	--
Sorghum	--	--	--	--	78.0
Minerals & vitamins	12.0	3.0	12.0	3.0	5.0
Protein level (%)	41.0	47.0	44.0	52.0	15.5
Daily consumption (kg)					
Cassava root silage	2.85	3.01	3.10	2.98	--
Protein supplement	0.86	0.67	0.73	0.60	--
Control diet	--	--	--	--	2.06
Performance of pigs					
Daily weight gain (kg)	0.59	0.55	0.59	0.50	0.56
Feed conversion ratio (DM)	3.27	3.31	3.24	3.50	3.31

Source*: Buitrago et al., 1978.*

The addition of 2% common salt (**Table 11**) to the cassava root silage showed a beneficial effect on feed conversion rate, without affecting the weight performance of pigs. The same experimental work demonstrated that silage stored for long periods (more than six months) does not affect production performance of pigs. The ensiled product progressively decreases in moisture content which resulted in better feed conversion ratios.

Table 11. The effect of feeding ensiled cassava roots with different storage time and added salt to Yorkshire growing-finishing pigs (22-95 kg) on their performance.

Age of silage	Salt addition	Silage consumption	Supplement consumption[1)]	ADG[2)]	FCR[3)]
> 6 Months	-	3.30	0.78	0.63	3.34
	2 %	2.87	0.78	0.62	3.10
< 6 Months	-	3.45	0.78	0.63	3.46
	2 %	3.20	0.78	0.63	3.27

[1)] 40% protein supplement with the following composition: 44% soybean meal, 44% cottonseed meal, 12% minerals & vitamins
[2)] Average daily weight gain
[3)] Feed conversion ratio
Source: *Buitrago et al., 1978.*

Ensiled Cassava Roots for Lactating Sows

In a similar experimental comparison as the one described for fresh cassava roots, ensiled cassava roots were also included in diets for lactating sows. Protein supplemented cassava silage diets were compared with corn-SBM diets, either fed as mixed or separated products (**Table 12**).

Performance of sows and litters was not affected by the use of cassava silage as total replacement of the cereal grains normally used in the dry lactation feeds. Even though the amount of cassava silage was more than twice the amount of dry feeds consumed by the sows, a small shortage of DM and energy is still observed in their total daily consumption. However the performance of sows and their litters was not affected up to weaning time. Litter size, individual weights as well as total litter weight were comparable among treatments, which demonstrate the feasibility for the inclusion of cassava root silage as the main component for lactating sows (**Table 12**).

PERFORMANCE RESULTS WITH FRESH CASSAVA ROOTS IN RUMINANT FEEDING

Fresh Cassava Roots for Dairy Cattle

Tables 13 and **14** show the effect on the performance of heifers and milking cows when the feeding treatments were mainly based on fresh cassava roots and protein supplements.

Heifers fed with cassava roots and protein supplement, in addition to green forage (sugarcane tops), showed a slightly superior daily weight gain than heifers receiving a

commercial concentrate based on conventional sources and the same green forage (**Table 13**).

Table 12. Ensiled cassava roots (ECR) and protein supplement for Yorkshire lactating sows.

	ECR + Supplement	Corn + Supplement	Mixed Corn-SBM
Feed ingredients (%)			
Corn	--	--	78.1
Soybean meal	78.0	56.0	16.4
Minerals & vitamins	22.0	44.0	5.5
Protein level (%)	**40**	**28**	**16**
Daily feed consumption of sows (kg)			
Cassava silage	9.35	--	--
Corn	--	4.27	--
Protein supplement	1.11	0.66	--
Complete diet (Corn-SBM)	--	--	4.54
Performance of sows			
Weight at farrowing, kg	140.9	168.5	155.4
Weight at weaning (35 days), kg	151.2	182.3	179.7
Performance of litters at birth			
Number of piglets	10.6	10.0	10.7
Individual weight, kg	1.09	1.16	1.12
Total litter weight, kg	11.50	11.60	12.04
Performance of litters at weaning (35 days)[1)]			
Number of piglets	8.22	7.00	8.11
Individual weight, kg	5.54	4.95	5.33
Total litter weight, kg	45.51	34.66	43.23

[1)] Piglets consumed the same creep feed at free choice.
Source: *Buitrago et al., 1978.*

Confined milking cows also showed a slightly superior production of milk associated with the consumption of cassava roots and protein supplement in addition to star grass hay (**Table 14**).

Fresh Cassava Roots for Beef Cattle

The results of a feedlot study are shown in **Table 15** in which growing-finishing steers were supplemented with a fixed level of fresh grass (elephant grass) plus different dry supplements vs. the cassava group which was fed a similar quantity of fresh grass plus fresh cassava roots and a protein supplement with a high level of urea. One part of the protein supplement was mixed with 10 parts of cassava roots as a complement to the fresh grass in this last group.

The performance results demonstrated excellent growing rates and feed efficiency in the cassava fed group. The inclusion of a high level of urea in the cassava group provides an important advantage by replacing a high percentage of other costly protein sources.

Table 13. Fresh cassava roots and protein supplementation in Holstein growing heifers[1)].

	Commercial concentrate	Fresh cassava roots + protein supplement
Ingredients for supplemental feeding (%)[2)]		
Corn	59.00	--
Sugarcane molasses	10.0	12.0
Wheat bran	14.0	16.3
Cottonseed meal	13.0	61.0
Urea	1.5	3.7
Minerals and vitamins	2.5	7.0
Daily consumption (kg)		
Commercial concentrate	2.64	--
Protein supplement	--	1.08
Cassava roots (DM) [3)]	--	1.56
Sugarcane tops (DM) [3)]	4.82	4.17
Total DM intake (kg)	**7.46**	**6.81**
Performance of heifers		
Initial weight, kg	191.8	190.6
Final weight, kg	366.8	377.3
Daily weight gain, kg	0.78	0.83

[1)] Heifers on group confinement from 8 to 16 months.

[2)] Heifers in the control group received 3 kg of commercial concentrate per day.
Heifers in the cassava group received 4.5 kg of fresh cassava and 1.23 kg of protein supplement per day.
Besides the supplemental feed all heifers received fresh sugarcane tops *ad libitum*

[3)] Daily consumption expressed as dry matter (DM).

Source: *Pineda and Rubio, 1972.*

PERFORMANCE RESULTS WITH FRESH CASSAVA FOLIAGE IN RUMINANT FEEDING

The use of fresh cassava foliage is almost limited to ruminant feeding, considering its high moisture (70-72%) and fiber (4-6%) levels. Due to its high quantity and quality of protein, the fresh product resembles conventional legumes and is suitable as a forage supplement for ruminants.

The best quality foliage should contain a larger proportion of green leaves, petioles or tender parts from branches, and a minimum of stems or woody parts of the plant. The age of the plant is also an important factor in defining the nutritional quality: when cuts are made from the early stage forage (i.e. less than 3 months) and thereafter harvested at frequent intervals (i.e. every 2-3 months), an excellent product can be obtained in terms of nutrient quality and quantity.

Special care should be taken with fresh forage due to the higher level of HCN in leaves and petioles. The chopping or cutting procedure plus a wilting process during at least 6 hours is very effective in reducing the HCN concentration to safe levels in cattle feeding.

Table 14. Fresh cassava roots and protein supplementation in White Fulani milking cows[1)].

	Commercial concentrate	Fresh cassava roots + protein supplement
Ingredients for supplemental feeding (%)[2)]		
Corn	50.0	--
Palm cake	40.0	50.0
Peanut cake	10.0	50.0
Nutrient content (%)		
DM	90.0	91.0
Protein	15.7	26.7
Fiber	5.3	6.6
Fat	4.9	9.7
Performance of heifers		
4 % fat corrected milk (kg)	6.8	7.2

[1)] Confined cows during an 84-days lactation period.

[2)] Cows in the control group received 0.42 kg of concentrate per kg of milk produced. Cows in the cassava group received 0.75 kg of fresh cassava roots plus 0.20 kg of protein supplement per kg of milk produced.

Besides the supplemental feed all cows received star grass hay.

Source: *Olaloku et al., 1971.*

Tables 16, 17 and **18** illustrate three examples with dairy and beef cattle where cassava foliage is included in a large proportion of their feeding program. In all cases there was an improvement in animal performance associated with the inclusion of cassava foliage. In one of the trials, cassava foliage was offered as a total replacement of alfalfa forage with superior performance results for this treatment (**Table 16**). **Table 18** also illustrates that higher inclusion levels of cassava foliage resulted in a linear increase of weight gains and improvement of the feed efficiency in fattening steers.

Table 15. Fresh cassava roots and protein supplementation in growing finishing Gyr x Brown Swiss steers[1)].

	Cassava + Supplement	Commercial concentrates		
Ingredients (%)				
Corn	--	34.0	--	--
Rice polishings	--	53.0	--	--
Cottonseed meal	75.0	10.0	16.3	15.3
Corn husks	--	--	81.4	--
Cottonseed husks	--	--	--	82.4
Urea	12.0	--	--	--
Minerals and vitamins	13.0	2.3	2.3	2.3
Nutrient content (%)				
Protein	64.65	13.95	9.58	9.09
NDT	45.0	63.0	50.0	48.0
Ca	4.1	0.93	0.74	0.82
P	1.02	0.98	0.93	0.94
Daily feed consumption (kg)[2)]				
Elephant grass	9.8	9.8	9.8	9.8
Fresh cassava	15.8	--	--	--
Protein supplement	1.6	--	--	--
Commercial concentrate	--	8.9	5.6	9.6
Total DM intake	8.4	9.3	6.4	9.9
Performance of steers				
Initial weight, kg	252	252	252	252
Final weight, kg	402	432	346	359
Daily weight gain, kg	1.39	1.66	0.87	0.99
Carcass yield, %	56.7	54.0	46.0	50.4

[1)] 22-24 month old steers.

[2)] Cassava roots were supplied at free choice in a 10:1 ratio with the protein supplement. Commercial feeds were supplied at free choice.

Source: *Terleira et al., 1975.*

Table 16. Fresh cassava foliage as a complement to grazing Holstein heifers[1)].

Daily consumption (kg/animal/day)		
Fresh cassava foliage	7.50	--
Fresh alfalfa	--	10.00
Cane molasses	0.50	0.50
Mineral salt	*Ad libitum*	*Ad libitum*
Performance of heifers		
Initial weight, kg	189.3	183.6
Final weight, kg	256.3	241.3
Daily weight gain, kg	0.68	0.59

[1)] Growing heifers on star pangola grazing lots.
Source: *Zapata et al., 1985.*

Table 17. Fresh cassava foliage and elephant grass for crossbred Zebu finishing steers on group confinement[1)].

Feed mixture for free choice consumption			
Elephant grass, % of mixture [2)]	100	75	50
Cassava foliage, % of mixture	--	25	50
Performance of steers			
Initial weight, kg	265.5	276.3	270.0
Final weight, kg	342.5	392.7	379.0
Daily weight gain, kg	0.31	0.46	0.44
Feed conversion rate	17.6	13.7	13.7

[1)] Growing steers on group confinement.
[2)] Fresh mixture offered for free choice consumption.
Source*: Moore, 1976.*

Table 18. Fresh cassava foliage and molasses for crossbred Zebu finishing steers on group confinement[1)].

	Forage consumption as % of body weight (kg/day)		
	2 %	3 %	4 %
Fresh cassava foliage	3.70	5.50	7.35
Free choice cane molasses	3.78	3.61	4.29
Performance of steers			
DM consumption per day, kg	3.86	4.13	7.35
Daily weight gain, kg	0.37	0.47	0.91
Feed conversion rate	10.7	8.78	5.61

[1)] Finishing steers on group confinement; ***Source:*** *Fernández and Preston, 1978.*

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CHAPTER 26

DRY CASSAVA ROOT AND FOLIAGE MEAL FOR POULTRY, SWINE AND RUMINANTS [1]

Julián Buitrago A., DVM, PhD. [2]

The information concerning the use of dried cassava products for animal feeding is quite ample in all productive species, mainly swine, poultry and ruminants. The dried products can be handled more easily and with higher accuracy than programs based on fresh or ensiled cassava.

The roots and foliage are dehydrated in order to increase the total nutrient concentration and to facilitate the preservation of the finished feed. In addition, dehydration by heat eliminates most of the cyanogenic components which produce toxic and deleterious effects on animal performance.

Cassava root meal is essentially a carbohydrate product with a high concentration of starch (60-65%). The metabolizable energy content of good quality meal for poultry and swine is around 3.20 and 3.40 Mcal/kg, respectively, while the total digestible nutrient (TDN) content is around 86%. Its main nutritional limitation is due to the low protein level, so that protein supplementation is required, with special emphasis on the first limiting aminoacid: methionine.

The quality of the roots being dehydrated to produce cassava root meal has a natural, direct influence on the final quality of the product. Roots with fibrous impurities (stems, leaves, peels, waste material) or those contaminated with sand or soil affect the nutritional quality and reduce the energy concentration.

Although there is not an official method to grade the quality of cassava root meal, **Table 1** shows an approach, based on the proposal of Muller *et al*. (1972), and complemented by the author of this paper. This initiative refers principally to the parameters of primary importance for determining the energetic value (principal nutrient of the roots), and giving a secondary value to the elements of lower concentration in the root (protein, aminoacids).

Based on the above classification, it is possible to recommend the use of cassava root meal, according to more precise nutritional criteria, and better adapted to the different animal production stages, as follows:

Grade 1: broilers, piglets and aquaculture.
Grades 1 and 2: layers, growing-finishing pigs, calves.
Grades 1, 2 and 3: pullets, gestating and lactating pigs.
Grades 1, 2, 3 and 4: dairy, beef, goats, horses.

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[1] For color photos see pages 790-793.

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Table 1. Quality grading of cassava root meal based on energy concentration.

Grade	Raw fiber (%)	Ash (%)	Fiber + Ash (%)	Metabolic energy (Mcal/kg)
1	< 2.8	< 2.0	< 4.8	3.30
2	< 3.6	< 2.5	< 6.1	3.15
3	< 4.5	< 3.2	< 7.7	2.92
4	< 5.2	< 4.0	< 9.2	2.60

Source: *Buitrago, 1990*.

Conversely, cassava foliage meal is characterized by its high fiber and protein levels. Depending on the leaves:stems ratio and the age of the plant, crude fiber may range between 10 and 30%, while the protein content may vary from 16 to 28%. Under practical conditions, the green plant top or its third superior aerial part, should be considered as the recommended material to be processed.

The plant top is a mixture of leaves, petioles and primary and secondary stems. The proportion in which these elements participate in the final product will determine the nutritional quality of the foliage meal. **Table 2** illustrates the differences in separate samples of the foliage components.

Table 2. Nutritional composition of cassava foliage meal with different proportions of leaves, petioles and stems [1)].

Nutrients, %	Leaves	Leaves and petioles	Leaves, petioles and stems
Protein	22.7	21.6	20.2
Ash	10.9	9.8	8.5
Fat	6.3	6.3	5.3
Fiber	11.0	11.6	15.2
Calcium	1.68	1.70	1.68
Phosphorus	0.29	0.24	0.28
Potassium	0.69	0.60	1.09

[1)] Products with 8-10% humidity

Source: *Van Poppel, 2001.*

Different alternatives may be considered when foliage tops are harvested for feeding purposes: a single cut may be obtained simultaneously with the root at harvesting time, or the tops may be cut periodically (every 2-3 months) without root harvesting. Moreover, the cassava crop can be completely oriented for just foliage production.

It is also important to note that foliage meal from early regrowth (less than 3 months) will provide better nutritional characteristics (more than 18% protein and less than 20% fiber) in contrast with late regrowths (less than 18% protein and more than 20% fiber) as is illustrated in **Table 3**.

Table 3. Nutritional composition of cassava foliage meal at different harvesting times.

Main nutrients	Cassava foliage meal[1)]		
	2-3 Months	5-6 Months	More than 8 months
Protein, % of DM	22.0	18.0	16.0
Fiber, %	16.0	20.0	26.0
Ash, %	5.5	5.8	5.8
Fat, %	5.2	5.6	5.6
Calcium, %	1.6	1.7	1.7
Phosphorus, %	0.26	0.28	0.28
TDN[2)],%	68.0	66.0	58.0
DE[2)], Mcal/kg	2.94	2.65	2.40

[1)] Third superior top (including leaves, petioles and young stems)
[2)] TDN = total digestible nutrients; DE = digestible energy
Source: *Buitrago, 1990.*

PERFORMANCE RESULTS WITH DRIED CASSAVA ROOTS IN POULTRY FEEDING

The results of some selected experiences will be presented in the following tables, where cassava root meal is included in medium to high levels of the diet for broilers and layers. Most of the early demonstrations were conducted with meal type (ground) diets and free choice consumption. In the more recent experiences, pelletized diets were introduced as an important mechanism to improve the performance of broilers and to reduce the dusty conditions in diets with high cassava meal content.

The economic considerations when cassava root meal replaces corn or other cereal grains in commercial operations should consider the lower energy and protein values of the cassava root. These limitations normally indicate that cassava root meal should have a cost not higher than 70 to 80% of the price of corn.

Dried cassava root meal for broilers

Table 4 illustrates an early study to measure the effect of diets where cassava meal gradually replaced corn as the energy source for broiler diets, without the adjustment of energy level. The results show a slight decrease in performance mainly associated with higher levels of cassava meal due to the reduction in metabolizable energy.

Table 4. Different levels of cassava root meal in diets for broilers [1].

	Cassava content							
	0		15 %		30 %		45 %	
Ingredients (%)	S [2]	F [2]	S	F	S	F	S	F
Cassava root meal	0	0	15.0	15.0	30.0	30.0	45.0	45.0
Corn	59.9	64.0	42.9	47.4	26.3	30.7	9.7	14.1
Soybean meal	30.7	27.6	31.0	28.2	32.0	29.0	33.0	30.0
Fish meal	6.0	4.0	7.3	5.0	7.9	5.8	8.5	6.5
DL-methionine	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Minerals & vitamins	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
Nutritional content								
ME, Mcal /kg	2.94	2.96	2.86	2.89	2.78	2.79	2.70	2.72
Protein, %	22.1	20.0	22.1	20.0	22.1	20.0	22.1	20.0
Methionine + Cystine, %	0.87	0.80	0.87	0.79	0.86	0.80	0.86	0.79
Lysine	1.26	1.10	1.33	1.26	1.39	1.22	1.44	1.28
Performance of broilers								
Final weight, kg	1.47		1.50		1.45		1.39	
Feed consumption, kg	3.33		3.39		3.48		3.29	
Feed conversion ratio[3]	2.45		2.42		2.56		2.56	

[1] 0-8 weeks broilers

[2] S:starting: 0-5 weeks; F: finishing: 5-8 weeks

Source: *Vasquez et al., 1977.*

Table 5. Different levels of cassava root meal in iso-energetic diets for broilers [1].

	Cassava meal level (%)					
	0	20	30	40	50	58
Ingredients (%)						
Cassava root meal	0	20.0	30.0	40.0	50.0	58.0
Corn	54.0	30.0	16.0	9.0	3.9	-
Rice polishing	10.0	9.0	8.6	8.1	0	-
Fish meal	6.0	6.0	6.0	6.0	10.0	11.0
Soybean meal	27.0	31.0	35.0	32.0	32.0	27.0
Vegetable oil	-	1.0	1.4	1.9	2.0	2.0
Minerals & vitamins	3.0	3.0	3.0	3.0	2.1	2.0
Broiler performance						
Final weight, kg	2.04	2.05	2.04	2.03	2.04	2.04
Feed conversion ratio	2.61	2.59	2.64	2.61	2.56	2.53
Mortality, %	9.2	3.0	3.0	4.0	10.2	5.0

[1] 0-6 week broilers.

Source: *Chou et al., 1973.*

The inclusion of vegetable oil in diets with high cassava meal compensates the lower energy and provides an improvement in performance of broilers, as is illustrated in **Table 5**, where the diets contained different levels of cassava meal but similar protein and metabolizable energy concentrations. In addition, vegetable oil provides an increment in linoleic acid, which is an essential fatty acid for poultry. Total replacement of corn by cassava meal did not affect body weight or feed conversion of broilers.

Pelletized diets provided an additional benefit to high cassava meal diets at the different levels of cassava meal inclusion for broiler diets **(Table 6).**

Table 6. Different levels of cassava root meal in pelletized iso-energetic diets for broilers [1)].

	Cassava meal level (%)					
	0	10	20	30	40	50
Ingredients (%)						
Cassava root meal	0	10.0	20.0	30.0	40.0	50.0
Wheat	53.9	48.9	38.9	28.8	18.3	6.1
Corn	16.2	10.5	9.5	9.0	9.0	10.0
Soybean meal	16.3	14.8	13.8	12.8	11.6	11.1
Fish meal	5.0	6.8	8.9	10.5	11.4	12.5
Meat meal	3.0	3.0	3.0	3.1	4.3	5.0
Vegetable oil	3.1	3.9	3.9	3.9	3.6	3.5
DL-methionine	0.11	0.12	0.15	0.18	0.20	0.23
Minerals & vitamins	2.4	2.0	1.9	1.7	1.6	1.4
Nutritional composition						
ME, Megajoules/kg[2)]	13.7	13.5	13.8	13.9	13.9	13.8
Protein, %	19.3	19.7	20.0	19.4	19.4	19.8
Broiler performance						
Final weight, kg	2.31	2.39	2.30	2.31	2.31	2.30
Feed consumption, kg	4.45	4.49	4.39	4.59	4.38	4.62
Feed conversion ratio	1.92	1.88	1.91	1.99	1.90	2.01

[1)] 0-7 week broilers.

Source: *Stevenson and Jackson, 1983.*

Dried cassava root meal for layers

The inclusion of dried cassava roots in layer feeding has also been experimented in different comparisons where corn is gradually replaced. In several of the earlier studies there was not a precise adjustment in some of the nutrients, mainly metabolizable energy,

methionine and linoleic acid (**Tables 7, 8 and 9**), which lowers the production performance.

Table 7. Performance of Leghorn layers with increasing levels of cassava root meal [1].

Ingredients (%)			
Cassava root meal	--	29.5	59.0
Corn	59.0	29.5	--
Soybean meal	16.6	19.4	22.2
Fish meal	8.0	8.0	8.0
Coconut cake	5.0	5.0	5.0
Sand	5.7	2.9	0.13
Vitamins & minerals	5.6	5.6	5.6
Nutritional composition			
ME, Mcal/kg	2.74	2.69	2.63
Protein, %	18.5	18.5	18.5
Calcium, %	3.08	3.24	3.40
Phosphorus, %	0.72	0.83	0.94
Performance of layers			
Egg production, %	70.9	66.6	69.3
Daily feed consumption, g	103	114	116
Feed conversion ratio [2]	2.88	3.37	3.30

[1] 20-32 week layers
[2] kg feed consumption/kg eggs produced
Source: *Vasquez et al., 1977.*

Egg production and feed conversion ratio are affected in most cases when cassava meal replaces corn without adjustments in the diet, especially at high levels of substitution. Egg yolk pigmentation is also affected with high levels of cassava root meal due to the absence of xantophyl pigments in roots, in contrast with its high concentration in cassava leaves.

Once the nutrient adjustments are introduced in diets with high levels of cassava root meal, improvement on production parameters are generally obtained. The essential aminoacid methionine and the energy concentration are important factors in egg production and egg size, while linoleic acid is mainly involved in egg size. **Tables 10** and **11** illustrate the effect of high levels of cassava root meal when the diets are correctly balanced in energy and methionine. The results obtained in egg production, egg size and feed conversion ratio are generally comparable with corn-soybean meal diets. The use of fullfat soybeans (8% linoleic acid) shows a favorable effect in the size, pigmentation and weight of eggs (**Table 11**).

8. Performance of Leghorn layers with increasing levels of cassava root meal [1].

Ingredients (%)					
Cassava root meal	--	5.0	10.0	50.0	60.0
Corn	77.0	69.5	63.5	18.0	10.0
Soybean meal	10.0	13.0	14.5	19.0	9.0
Fish meal	5.0	4.5	4.0	5.0	13.0
Vitamins & minerals	8.0	8.0	8.0	8.0	8.0
Nutritional composition					
ME, Mcal/kg	3.16	3.14	3.13	3.14	3.20
Protein, %	15.2	15.8	15.7	15.3	15.2
Methionine + cystine, %	0.60	0.66	0.67	0.59	0.49
Lysine, %	0.70	0.85	0.86	0.97	1.05
Performance of layers					
Egg production, %	67.62	60.36	58.03	47.32	57.77
Weight of eggs, g	56.03	52.61	53.08	56.12	52.94
Daily feed consumption, g	94	92	93	107	98

[1] 32-42 week layers

Source: *Jalaludin and King, 1973.*

Table 9. Performance of Leghorn layers with increasing levels of cassava root meal [1].

Ingredients (%)				
Cassava root meal	--	10.0	25.0	50.0
Corn	62.0	50.0	32.1	2.1
Soybean meal	9.20	11.20	14.1	19.1
Rice bran	5.0	5.0	5.0	5.0
Copra meal	7.5	7.5	7.5	7.5
Fish meal	5.0	5.0	5.0	5.0
Meat and bone meal	2.5	2.5	2.5	2.5
Leucaena meal	3.0	3.0	3.0	3.0
Vitamins & minerals	5.8	5.8	5.8	5.8
Performance of layers				
Egg production, %	63.9	62.8	58.7	62.8
Weight of eggs, g	58	57	57	57
Feed conversion ratio	2.01	2.10	2.22	2.12
Yolk pigmentation[2]	6.0	6.0	5.0	3.5

[1] 20-48 week layers

[2] Roche pigmentation scale.

Source*:Enriquez and Ross, 1972.*

Table 10. Performance of Hisex layers with increasing levels of cassava root meal [1].

Ingredients (%)						
Cassava root meal	--	10.0	20.0	30.0	40.0	50.0
Wheat	50.0	50.0	46.1	30.8	15.5	--
Corn	13.2	8.5	5.8	8.8	11.9	15.2
Barley	12.7	5.4	--	--	--	--
Fish meal	3.0	3.0	3.0	3.0	3.0	2.9
Soybean meal	7.9	9.9	11.9	14.2	16.5	18.8
Meat and bone meal	5.0	5.0	5.0	5.0	5.0	5.0
Animal fat	1.0	1.0	1.0	1.0	1.0	1.0
DL-methionine	0.05	0.06	0.07	0.08	0.09	0.09
Vitamins & minerals	7.2	7.1	7.1	7.0	7.0	6.9
Nutritional composition						
ME, MJ/kg	11.0	11.0	11.0	11.0	11.5	11.1
Protein, %	15.9	15.7	15.9	15.8	15.9	16.0
Calcium, %	3.2	3.3	3.3	3.3	3.3	3.3
Phosphorus, %	0.64	0.63	0.63	0.60	0.58	0.57
Performance of layers						
No. eggs in 280 days	205	203	205	215	201	196
Weight of eggs, g	55	56	55	55	55	56
Daily feed consumption, g	119	119	111	113	112	109
kg of eggs/kg of feed	0.38	0.34	0.35	0.38	0.35	0.36

[1] 27-67 week layers.

Source: *Stevenson, 1984.*

Dried cassava root meal for replacement pullets

Most feeding experiences with dry cassava roots in replacement pullets (young hens) have indicated that partial or total substitution of corn and other grain cereals by cassava root meal does not have a negative effect on performance of pullets during early or late growing stages (**Tables 12** and **13**), even though in most cases the small adjustment in methionine and energy levels have not been considered (**Tables 14**, **15**). However, there is also some information on a small negative effect when cassava meal is included at levels above 30-40 % (**Tables 16** and **17**).

Table 11. Performance of Shaver layers with increasing levels of cassava root meal[1)].

Ingredients (%)						
Cassava root meal	--	25.0	50.0	--	25.0	50.0
Sorghum	65.2	38.7	12.1	57.3	28.3	--
Soybean meal	11.3	14.8	18.3	--	--	--
Fullfat soybeans	--	--	--	15.3	20.0	24.7
Fish meal	7.0	7.0	7.0	7.0	7.0	7.0
DL-methionine	0.13	0.16	0.18	0.14	0.17	0.19
L-lysine	0.17	0.10	0.04	0.15	0.08	--
Corn cobs	6.6	4.9	3.2	10.6	10.1	6.8
Vitamins & minerals	9.6	9.3	9.2	9.5	9.3	11.3
Nutritional composition						
ME, Mcal/kg	2.65	2.65	2.65	2.65	2.65	2.65
Protein, %	15.5	15.5	15.5	15.5	15.5	15.5
Methionine + cystine, %	0.66	0.66	0.66	0.66	0.66	0.66
Lysine, %	0.98	0.98	0.98	0.98	0.98	0.98
Linoleic acid, %	0.78	0.51	0.24	1.92	2.01	2.10
Performance of layers						
Egg production, %	72.3	77.9	78.0	72.6	72.0	74.5
Weight of eggs, g	69	67	67	70	71	69
Daily feed consumption, g	125	133	132	122	121	120
Yolk pigmentation[2)]	5.1	4.9	4.7	6.4	6.5	6.3

[1)] 42-62 week layers

[2)] Roche pigmentation scale.

Source: *Hennesey and Ayala, 1986.*

Table 12. Total substitution of cereal grains by cassava root meal in replacement pullets [1)].

Ingredients (%)				
Cassava root meal	--	--	--	60.0
Sorghum	53.4	--	--	--
Corn	--	53.4	--	--
Rice	--	--	52.0	--
Soybean meal	15.2	15.2	16.6	28.0
Fish meal	3.0	3.0	3.0	3.0
Rice bran	25.0	25.0	25.0	5.6
Vitamins & minerals	3.4	3.4	3.4	3.4
Nutritional composition				
ME, Mcal/kg	2.61	2.67	2.52	2.60
Protein, %	16.8	16.1	16.3	16.1
Performance of pullets				
Initial weight, g	621.0	710.0	621.0	689.0
Final weight, g	1,475	1,513	1,475	1,478
Feed/weight gain ratio	4.21	4.64	4.22	4.63

[1)] 7-16 week pullets

Source: *Phalarksh et al., 1978.*

Table 13. Total substitution of cereal grains by cassava root meal in replacement pullets [1].

Ingredients (%)				
Cassava root meal	--	--	--	60.0
Sorghum	54.35	--	--	--
Corn	--	53.35	--	--
Rice	--	--	53.0	--
Soybean meal	7.50	9.25	8.85	21.0
Fish meal	3.0	4.0	4.0	4.0
Rice bran	26.75	29.0	25.75	6.6
Animal fat	1.0	--	1.0	1.0
Dehydrated grass	4.0	--	4.0	4.0
Vitamins & minerals	3.4	3.4	3.4	3.4
Nutritional composition				
ME, Mcal/kg	2.66	2.62	2.62	2.65
Protein, %	14.6	14.6	14.5	14.6
Performance of pullets				
Initial weight, g	1,601	1,563	1,629	1,547
Final weight, g	1,823	1,765	1,849	1,766
Feed/weight gain ratio	6.22	6.98	5.68	7.71

[1] 16-20 week pullets

Source: *Phalarksh et al., 1978.*

Table 14. Partial substitution of sorghum by cassava root meal in replacement pullets [1].

Ingredients (%)				
Cassava root meal	--	10.0	20.0	30.0
Sorghum	71.8	60.2	48.4	36.5
Soybean meal	20.0	21.7	23.5	25.5
Cane molasses	5.0	5.0	5.0	5.0
Bone meal	2.5	2.5	2.5	2.5
L-lysine	0.13	0.09	0.05	--
Vitamins & minerals	0.50	0.5	0.5	0.5
Performance of pullets				
Final weight, kg	1.29	1.26	1.30	1.29
Total feed consumption, kg	4.06	3.82	3.96	3.84
Mortality, %	0.6	0.10	0.40	0.70

[1] 9-16 week pullets

Source: *Santos et al., 1985.*

Table 15. Total substitution of corn by cassava root meal in two lines of replacement pullets [1].

Ingredients (%)		
Cassava root meal	--	50.0
Corn	55.9	--
Rice bran	33.0	32.9
Soybean meal	5.0	11.0
Fish meal	2.5	2.5
Vitamins & minerals	3.6	3.6
Performance of pullets		
Performance (Golden Comet)		
Initial weight, g	1,054	1,052
Total weight gain, g	591	589
Feed/weight gain ratio	7.8	7.9
Performance (Shaver)		
Initial weight, g	927	934
Total weight gain, g	407	415
Feed/weight gain ratio	10.5	10.2

[1] 12-21 week pullets: Golden Comet and Shaver lines.
Source: *Chou et al., 1973.*

Table 16. Gradual substitution of corn by cassava root meal in replacement pullets [1].

Ingredients (%)				
Cassava root meal	--	10.0	25.0	50.0
Corn	61.2	49.2	31.2	1.2
Soybean meal	6.0	8.0	11.0	16.0
Rice bran	10.0	10.0	10.0	10.0
Copra cake	10.0	10.0	10.0	10.0
Fish meal	4.0	4.0	4.0	4.0
Meat and bone meal	4.0	4.0	4.0	4.0
Leucaena meal	3.0	3.0	3.0	3.0
Vitamins & minerals	1.8	1.8	1.8	1.8
Performance of pullets				
Final weight, g	935	1007	925	808
Total feed consumption, kg	6.02	6.45	6.40	5.95
Feed/weight gain ratio	6.43	6.30	6.69	7.37

[1] 6-20 week pullets.
Source: *Enriquez and Ross, 1972.*

Table 17. Gradual substitution of corn by cassava root meal in replacement pullets [1)].

Ingredients (%)				
Cassava root meal	--	15.0	30.0	45.0
Corn	70.7	52.8	34.8	16.8
Peanut meal	20.0	22.5	25.2	27.7
Fish meal	4.0	4.5	5.0	5.0
Wheat bran	2.0	2.0	2.0	2.0
DL-methionine	0.06	0.07	0.08	0.08
L-lysine	0.23	0.18	0.13	0.13
Vitamins & minerals	3.1	3.1	3.1	3.1
Nutritional composition				
ME, MJ/kg	12.8	12.8	12.8	12.8
Protein, %	19.6	19.0	19.3	19.7
Methionine + cystine, %	0.72	0.72	0.73	0.71
Lysine, %	1.03	1.03	1.04	1.04
Performance of pullets				
Daily weight gain, g	12.8	13.4	13.3	11.9
Total feed consumption, kg	45.2	51.1	50.0	50.0
Feed/weight gain ratio	3.53	3.82	3.76	4.2

[1)] 6-12 week Leghorn pullets.
Source: *Ademosun and Eshiett, 1980.*

PERFORMANCE RESULTS WITH DRIED CASSAVA ROOTS IN SWINE FEEDING

Several experiments have been conducted with swine in order to demonstrate the effect of different levels of cassava root meal in conventional feeding programs for piglets, growing, finishing, gestating and lactating pigs. Partial to total substitution of cereal grains, inclusion of different protein supplements and comparisons between sweet and bitter varieties of cassava have been analyzed in a large number of feeding trials.

As already mentioned in poultry feeding, with high levels of cassava meal the dustiness of the diet may become one of the main limitations for an efficient use of the mixed diet. The addition of sugarcane molasses, animal fat or vegetable oil helps in the prevention of the dusty presentation and to avoid feed waste. Whenever it becomes possible, pellet processing is the best practice when high levels of cassava meal have to be included.

Similarly to poultry feeding, the cost of cassava meal compared to corn or other cereal grains is the key factor in deciding the economics of its use. As mentioned earlier, the lower energy and protein concentration in cassava root meal generally bears to an adjustment in the price of cassava meal, which, in general, should be equivalent to around 70-80% of the price of corn.

Dried cassava root meal for growing-finishing pigs

Feeding practices with dried cassava roots have been extensively studied during the growing-finishing stage of pigs. Some of the most representative feeding studies have been

selected in the following tables, which summarize the performance results under different environmental and management conditions.

Table 18 compares sweet (less than 80 ppm HCN) and bitter (150-200 ppm HCN) varieties of cassava root meal as the main source of energy in diets for growing pigs. Although the sun drying process partially reduced the HCN content, there is still a negative effect in consumption and weight gains of the pigs. However, this effect is very marginal compared to the effect observed when the roots are fresh, since all HCN remains in the tissue of the unprocessed product.

Table 18. Bitter vs. sweet varieties of cassava root meal for growing Yorkshire pigs [1].

Ingredients (%)	Bitter[2]	Sweet[3]
Cassava root meal	71.0	71.0
Soybean meal	25.0	25.0
Vitamins & minerals	4.0	4.0
Performance of pigs		
Daily weight gain, kg	0.56	0.62
Feed consumption, kg	1.35	1.77
Feed conversion ratio	2.43	2.86

[1] 38-58 kg.
[2] CMC-84 variety with 150-200 ppm HCN
[3] 80 ppm HCN
Source: *Gómez and Buitrago, 1982.*

In most studies the inclusion of low HCN cassava root varieties can replace cereal grains without detrimental effects in growing-finishing pigs, even though in some trials no adjustments were made in the energy levels of high cassava diets (**Tables 19, 20** and **21**). Sorghum was included in two of these trials (**Tables 19** and **20**), while corn was used in the third trial (**Table 21**).

The studies in **Tables 20** and **21** also present information on carcass characteristics at slaughtering time. Yields of lean meat cuts were not affected and no clear differences were noticed on fat percentages, fat quality or saturation index (iodine number), although all animals showed a larger proportion of body fat proper to the crossbred pigs available at the experimental time.

The addition of cane molasses, raw sugarcane or animal fat to diets based on cassava root meal as the only energy source, did not contribute to the improvement of feed consumption or performance in growing pigs, as shown in **Table 22.** Animal fat addition decreased feed consumption and improved the feed conversion ratio, due to the increment in energy density of the diet. Unexpectedly, methionine supplementation did not improve the performance of growing pigs in this study. Nevertheless, in other experiments the beneficial effect of methionine supplementation to diets containing high levels of cassava has been observed.

Table 19. Low HCN cassava root meal varieties in substitution of sorghum for growing crossbred pigs [1) 2)].

Ingredients (%)					
Cassava root meal	--	18.6	35.8	52.0	67.0
Sorghum	77.5	55.8	35.8	17.3	--
Soybean meal	16.5	19.1	21.4	22.7	23.9
Fish meal	3.0	3.5	4.0	5.0	6.0
Vitamins & minerals	3.0	3.0	3.0	3.0	3.0
Nutritional composition					
DE, Mcal/kg	3.31	3.29	3.27	3.25	3.23
Protein, %	17.0	17.0	17.0	17.0	17.0
Methionine, %	0.31	0.29	0.28	0.29	0.29
Lysine, %	0.84	0.91	0.96	1.02	1.07
Performance of pigs					
Daily weight gain, kg	0.56	0.54	0.54	0.57	0.55
Daily feed consumption, kg	1.92	1.93	1.93	1.93	1.93
Feed conversion ratio	3.46	3.58	3.61	3.41	3.55

[1)] 19-50 kg growing pigs.
[2)] 36 ppm HCN in fresh roots.
Source: *Méndez and Zaragoza, 1980.*

Table 20. Root meal of low-HCN cassava varieties in substitution of sorghum for growing crossbred pigs and their effect of carcass characteristics [1) 2)].

Ingredients (%)					
Cassava root meal	--	20.0	39.0	56.5	72.1
Sorghum	84.0	61.0	38.6	18.0	--
Soybean meal	11.8	14.0	17.4	19.0	22.1
Fish meal	1.2	2.0	2.0	2.5	2.8
Vitamins & minerals	3.0	3.0	3.0	3.0	3.0
Nutritional composition					
DE, Mcal/kg	3.32	3.30	3.28	3.26	3.25
Protein, %	13.0	13.0	13.0	13.0	13.0
Methionine, %	0.24	0.23	0.22	0.21	0.21
Lysine, %	0.59	0.67	0.72	0.78	0.82
Performance of pigs					
Daily weight gain, kg	0.56	0.54	0.54	0.57	0.55
Daily feed consumption, kg	1.92	1.93	1.93	1.93	1.93
Feed conversion rate	3.46	3.58	3.61	3.41	3.55
Carcass characteristics					
Carcass yield, %	74.4	74.8	75.8	75.6	75.0
Dorsal fat, cm	2.89	3.27	2.52	2.53	2.73
Loin area, cm^2	32.5	31.0	36.9	39.4	38.2

[1)] 50-90 kg finishing pigs
[2)] 35 ppm HCN in fresh roots.
Source: *Méndez and Zaragoza, 1980.*

Table 21. Root meal of low-HCN cassava varieties in substitution of corn for growing crossbred pigs and their effect on carcass characteristics [1) 2)].

Ingredients (%)				
Cassava root meal	--	20.0	40.0	58.5
Corn	60.0	40.0	20.0	--
Meat meal	5.0	5.5	6.0	6.5
Sesame meal	20.0	23.0	26.0	29.0
Rice polishings	9.0	5.5	2.0	--
Cane molasses	5.0	5.0	5.0	5.0
Vitamins & minerals	1.0	1.0	1.0	1.0
Performance of pigs				
Daily weight gain, kg	0.79	0.78	0.84	0.80
Feed conversion ratio	3.50	3.60	3.30	3.30
Carcass characteristics				
Carcass length, cm	74.0	72.1	73.0	74.0
Dorsal fat, cm	3.10	3.40	3.30	2.90
Iodine number	69.3	64.5	71.3	69.3

[1)] 40-82 kg growing- finishing pigs
[2)] 40 ppm HCN in fresh roots.
Source: *Chicco et al., 1972.*

Table 22. Effect of adding cane molasses, raw sugar or animal fat to diets based on cassava root meal for Landrace x Yorkshire pigs [1)].

Ingredients (%)					
Cassava root meal	65.9	65.7	55.5	55.5	55.5
Soybean meal	29.4	29.4	29.8	29.8	29.8
Cane molasses	--	--	10.0	--	--
Raw sugar	--	--	--	10.0	--
Animal fat	--	--	--	--	10.0
DL-methionine	--	0.2	--	--	--
Vitamins & minerals	4.7	4.7	4.7	4.7	4.7
Performance of pigs					
Daily weight gain, kg	0.71	0.68	0.69	0.68	0.63
Daily feed consumption, kg	1.94	1.88	1.89	1.84	1.59
Feed conversion ratio	2.73	2.76	2.74	2.70	2.53

[1)] 20-50 kg growing pigs. Isoproteic (16 %) diets.
Source: *Maner et al., 1978.*

Table 23 illustrates the positive response to methionine, compared to other sulfur sources in an effort to explore the effect of sulfur in cassava based diets with high levels of hydrogen cyanide.

Table 23. Effect of adding methionine and other sulfur sources to diets based on cassava root meal for Landrace x Yorkshire pigs [1].

Feed treatment	Performance of pigs		
	Daily weight gain, kg	Daily feed consumption, kg	Feed conversion ratio
Control diet (CD) [2]	0.67	1.81	2.43
CD + 0.2 % methionine	0.70	1.77	2.29
CD + 0.8 % sodium thiosulfate	0.61	1.58	2.32
CD + 0.2 % elemental sulfur	0.65	1.64	2.29

[1] 20-50 kg growing pigs

[2] 16% protein control diet based on cassava root meal (70%), soybean meal (25%) and vitamin-mineral mixture (5%).

Source: *CIAT, 1975.*

Dried cassava root meal for gestating and lactating sows

The continued use of high levels of cassava root meal has also been tried during gestation and lactation in order to evaluate its effects on the mothers and on their offspring. **Tables 24** and **25** summarize the results observed in performance of Yorkshire and Duroc x Yorkshire females during gestation and lactation, as well as in piglets during the lactating period.

In **Tables 24** and **25** a corn-based diet was compared with diets where the corn was completely replaced by cassava root meal. The 16% protein diets were offered in controlled quantities during gestation and at free choice during lactation. In general, there are no detrimental effects in performance due to cassava usage, although the first trial (**Table 24**) shows a smaller litter size with no differences in the individual weight of piglets. Conversely, **Table 25** shows no differences in litter size, individual piglet weight or total litter weight. The weight differential between breeding time and weaning time of females was not affected when cassava root meal totally replaced corn.

Dried cassava root meal for piglets

Creep feed for lactating piglets with increasing levels of cassava meal has been offered from 10 days up to weaning time. The first trial results during a lactation period of 30 days are summarized in **Table 26.** No differences were observed in performance of piglets with levels up to 20% of cassava root meal in the diet. Weight gains, feed consumption and feed conversion were equivalent to piglets receiving diets with corn. In a second feeding trial (**Table 27**) pelleted diets with 0, 20 and 40% cassava meal were compared in order to measure consumption of lactating piglets when fed at free choice up to weaning time at 56 days. There was a positive effect in feed consumption associated with higher levels of cassava meal. Palatability of the diet and performance of piglets were clearly improved with increasing amounts of cassava root meal, even though dustiness was greater in these diets.

Table 24. Cassava root meal vs. corn in diets for gestating and lactation sows [1].

Ingredients (%)		
Cassava root meal	--	67.0
Corn	76.4	--
Soybean meal	18.8	28.2
Vitamins & minerals	4.8	4.8
Nutritional composition (%)		
Protein	16.0	16.0
Methionine + cystine	0.55	0.47
Lysine	0.77	0.92
Performance of sows		
Breeding weight, kg	127.6	118.5
Farrowing weight, kg	160.6	146.1
Weaning weight, kg	153.9	159.6
Performance of litters at farrowing		
No. piglets	10.0	8.4
Individual weight, kg	1.09	0.97
Litter weight, kg	10.9	8.15
Performance of litters at weaning		
No. of piglets	9.4	6.6
Individual weight, kg	15.87	15.70
Litter weight, kg	149.18	103.62

[1] 56-day weaning time; ***Source:*** *Gómez et al., 1976.*

Table 25. Cassava root meal vs. corn in diets for lactating sows [1].

Ingredients (%)		
Cassava root meal	--	59.1
Corn	81.5	--
Cane molasses	--	10.0
Soybean meal	15.0	27.4
Vitamins & minerals	3.5	3.5
Nutritional composition (%)		
Protein	16.0	16.0
Methionine + cystine	0.52	0.44
Lysine	0.71	0.89
Performance of sows		
Farrowing weight, kg	179.3	170.6
Weaning weight, kg	190.3	183.0
Performance of litters at farrowing		
No. piglets	10.8	10.1
Individual weight, kg	1.18	1.22
Litter weight, kg	12.74	12.32
Performance of litters at weaning		
No. piglets	9.01	7.90
Individual weight, kg	6.08	6.80
Litter weight, kg	54.0	53.7

[1] 35-day weaning time; ***Source:*** *Maner et al., 1978.*

Table 26. Effect of partial substituting of corn by cassava root meal in lactating piglets [1].

Ingredients (%)			
Cassava root meal	--	10.0	20.0
Corn	59.6	49.0	38.0
Soybean meal	27.7	28.3	28.9
Dehydrated milk whey	10.0	10.0	10.0
Vitamins & minerals	2.7	2.7	2.7
Nutritional composition			
Protein, %	18.5	18.1	17.8
Lysine, %	1.12	1.12	1.12
Calcium, %	0.78	0.78	0.78
Phosphorus, %	0.59	0.59	0.59
Performance of piglets			
Daily weight gain, kg	0.38	0.37	0.39
Daily feed consumption, kg	0.68	0.60	0.60
Feed conversion ratio	1.63	1.62	1.64

[1] 7-18 kg piglets (30 days)

Source: *Ravindran et al., 1983.*

Table 27. Feed consumption in lactating piglets associated with increasing levels of dry cassava root meal in their feed [1].

Age of piglets (days)	Total feed consumption per litter (kg) [2]		
	0 cassava meal	20% cassava meal	40% cassava meal
14 - 42	1.8	3.0	12.4
42 - 56	14.7	26.2	39.1
14 - 52 (total))	16.5	29.2	51.5

[1] 1-56 day piglets.

[2] Free choice cassava-sorghum-soybean diets with 20% protein.

Source: *Gómez et al., 1981.*

When piglet diets were completely based in cassava root meal, there was a response to increasing levels in digestible energy by adding vegetable oil and to methionine supplementation (**Table 28**). Piglets consuming higher energy diets improved feed consumption and weight gains.

PERFORMANCE RESULTS WITH DRIED CASSAVA ROOTS IN RUMINANT FEEDING

Cassava root meal diets have been used at different stages of ruminant nutrition. A selection of experimental diets and production performance obtained in calves, milking cows and growing-finishing steers are included in the following tables.

Dried cassava root meal for calves

Tables 29 and **30** describe different feeding treatments with variable levels of cassava root meal for early feeding of calves. At low levels of cassava meal, performance

was maintained close to those of the corn or sorghum-based diets but levels higher than 25% usually produced a slight decrease in consumption and growth rate of calves. In both experiments calves were raised with cow milk until the third or sixth week, and from this moment until the fourth month the dry diet was provided at free choice plus forages (alfalfa hay or ensiled sorghum) at free choice.

Table 28. Vegetable oil and methionine supplementation to cassava root meal diets for lactating piglets [1].

Ingredients (%)						
Cassava root meal	52.5	45.8	52.4	45.7	52.3	45.6
Soybean meal	38.2	38.2	38.2	38.2	38.2	38.2
Palm oil	3.0	9.7	3.0	9.7	3.0	9.7
Rice bran	2.5	2.5	2.5	2.5	2.5	2.5
DL-methionine	0.24	0.24	0.32	0.32	0.40	0.40
Vitamins & minerals	3.5	3.5	3.5	3.5	3.5	3.5
Nutritional composition						
DE, MJ/kg	14.63	15.94	14.81	15.98	14.63	15.94
Protein, %	20.0	20.0	20.0	20.0	20.0	20.0
Methionine + cystine, %	0.73	0.73	0.81	0.81	0.89	0.89
Lysine, %	1.22	1.22	1.22	1.22	1.22	1.22
Performance of piglets						
Daily weight gain, g	129	197	124	228	156	205
Daily feed consumption, g	338	459	272	535	372	577
Feed conversion ratio	2.62	2.33	2.19	2.35	2.38	2.81

[1] 8-15 kg piglets (42 days)

Source: *Balogun and Fetuga, 1984.*

Table 29. Effect of partial substitution of corn by cassava root meal in the feed of dairy calves [1].

	Energy source in dry feed [2]		
	50% sorghum	25% sorghum 25% cassava meal	50% cassava meal
Performance of calves (kg)			
Initial weight	35.15	34.10	34.26
Final weight	89.0	92.4	81.03
Daily weight gain	0.48	0.52	0.42
Total feed consumption in 112 days (kg)			
Dry feed	109.3	108.2	82.0
Alfalfa hay	28.4	28.6	29.1
Milk	132.7	135.3	126.9

[1] 1-112 day Holstein calves. Only milk during the first 42 days and *ad libitum* dry feed plus alfalfa hay from day 42 to day 112.

[2] Dry feed also supplemented with protein, mineral and vitamin sources.

Source: *Peixoto, 1973.*

Table 30. Effect of partial substitution of corn by cassava root meal in the feed of dairy calves [1].

Ingredients (%)		
Cassava root meal	10.5	34.5
Corn	36.8	10.0
Wheat bran	15.8	15.0
Copra cake	13.2	12.5
Sesame meal	13.3	18.0
Cane molasses	5.2	5.0
Dry milk	5.2	5.0
Nutritional composition (%)		
NDT	78.6	78.5
Protein	16.6	16.7
Total consumption of dry feed (kg)		
Males	68.22	62.23
Females	70.46	59.51
Daily weight gain of calves (grams)		
Males	532	445
Females	442	364

[1] 1-120 day Jersey calves. Only milk during the first 21 days and *ad libitum* dry feed plus sorghum silage from day 21 to day 120.

Source: *Valdivieso, 1958.*

Dried cassava root meal for dairy cows

The results from two experiments with dairy cows are described in the following tables. **Table 31** presents results in milking cows where dried diets were supplied in addition to sorghum silage. The inclusion of cassava root meal in substitution of 50% of the sorghum in the dried feed did not affect milk production. Similar results were observed when cassava meal replaced oats as the main energy source of the dried supplement (**Table 32**).

Dried cassava root meal for growing-finishing steers

Steers under intensive grazing or under total confinement have also been included in experiments where cassava root meal has been used as a component of the dried feed supplements.

Table 33 shows the results with growing-finishing steers under intensive grazing (4.8 head/ha) conditions, supplemented with controlled quantities of dry feed based on cassava root meal, cane molasses, urea and blood meal. Animals with higher levels of cassava consumption showed a slight increase in daily weight gain.

Table 34 shows the results with feedlot steers consuming a controlled amount of sorghum silage plus a free choice of dry supplement based on cassava meal or sorghum. Daily feed consumption of the supplement decreased with increasing levels of cassava meal. Conversely, sorghum silage consumption was increased to fulfill the energy deficit.

Nevertheless, there was a negative effect on daily weight gains associated with lower supplement consumption as a result of increasing levels of cassava root meal in the diet.

Table 31. Effect of partial substitution of sorghum by cassava root meal in dairy cows [1].

Ingredients in dry diets (%)[2]		
Cassava root meal	--	27.0
Sorghum	54.0	27.0
Cottonseed meal	44.0	43.5
Urea	--	0.50
Salt	1.0	1.0
Minerals	1.0	1.0
Nutritional composition (%)		
TDN	69.0	67.4
Protein	15.7	15.7
Daily milk production (kg)		
Non-corrected milk	12.0	12.4
4% fat corrected milk	11.4	11.3

[1] 63 days lactation period.
[2] Daily supply of 0.42 kg of dried feed per kg of milk produced plus *ad libitum* sorghum silage.
[3] TDN = Total digestible nutrients.
Source: *Ribeiro et al., 1976.*

Table 32. Effect of partial substitution of oats by cassava root meal in the feed of dairy cows [1].

	Energy source in dry feed [2]		
	Oats	Oats + cassava meal	Cassava Meal
Ingredients (%)			
Cassava root meal	--	12.5	25.0
Oats	25.0	12.5	25.0
Peanut meal	20.0	25.0	25.0
Legumes hay	35.0	35.0	35.0
Wheat bran	20.0	20.0	20.0
Nutritional composition (%)			
TDN[3]	69.0	67.0	65.0
Protein	15.5	16.0	15.5
Daily milk production (kg)			
Non-corrected milk	6.97	7.20	7.84
4% fat corrected milk	7.81	7.91	7.84

[1] 140 days lactation period.
[2] Daily supply of 1 kg of dried feed per 3 kg of milk produced plus *ad libitum* para grass hay.
[3] TDN = total digestible nutrients.
Source: *Mathur et al., 1969.*

Table 33. Growing-finishing crossbred Zebu steers under intensive grazing supplemented with two levels of cassava root meal [1].

	Dry supplement (kg/animal/day)	
Cassava root meal	0.65	1.10
Cane molasses	4.5	4.5
Urea	0.23	0.25
Blood meal	0.22	0.22
Performance of steers (kg)		
Initial weight	336.0	336.0
Final weight	403.0	411.0
Daily weight gain	0.71	0.77

[1] Steers on intensive grazing (4.8 head/ha) plus controlled dry supplement.
Source: *Lozada and Alderete, 1979.*

Table 34. Feedlot crossbred Zebu steers under total confinement with free choice consumption of sorghum-cassava meal supplement and controlled sorghum silage [1].

Ingredients in dry supplement (%)					
Cassava root meal	--	20.5	41.0	61.5	82.0
Sorghum	88.5	66.4	44.3	22.2	--
Cottonseed meal	7.8	9.2	10.5	11.9	13.3
Urea	1.7	1.9	2.2	2.4	2.7
Vitamins & minerals	2.0	2.0	2.0	2.0	2.0
Performance of steers					
Initial weight, kg	302.7	306.2	317.2	305.8	315.4
Final weight, kg	424.4	425.1	427.2	412.4	404.3
Daily weight gain, kg	1.16	1.13	1.05	1.01	0.85
Dry supplement consumption, kg	10.2	9.3	8.6	8.1	6.9
Silage consumption, kg	3.1	5.0	5.5	5.2	5.2
Dry feed/weight gain	8.79	8.23	8.18	8.08	8.18

[1] Free choice supplement and controlled sorghum silage (1.5 kg/100 kg body weight).
Source: *Delgado et al., 1975.*

PERFORMANCE RESULTS WITH DRIED CASSAVA FOLIAGE IN POULTRY FEEDING

In general, dried cassava foliage does not have a significant potential for poultry feeding due to its low energy level and poor palatability. As it happens with other forage products, fiber is a limiting factor which dilutes the concentration of the essential nutrients, mainly energy and protein. Although the protein level in good quality dried cassava foliage is high (18–26%), the high fiber and low energy concentration limits its use to levels not higher than 10%. The aminoacid profile is characterized by the high lysine content (7.2 g/100 grams of crude protein) and the low methionine level (1.7 g/100 grams of crude protein).

An important factor in cassava foliage, relevant to poultry feeding, is its high content in xanthophyll pigments (500-600 mg/kg), which improves the pigmentation of skin in broilers and egg yolk in layers when used at levels between 5 and 8% of the diet.

The best quality forage meal contains a larger proportion of leaves and young stems which can be easily obtained from plants less than three months of age. The nutritional quality decreases as the plant gets older and the leaf:stem ratio changes to a lower proportion of young leaves.

Though hydrogen cyanide levels in dehydrated foliage are generally over 200 ppm, the low foliage percentage recommended for poultry and pigs usually does not present a danger of toxicity; however, in some cases, a high hydrogen cyanide content can affect the palatability of the diet, and, eventually, cause toxicity problems.

It is suggested than no more than 6% of forage meal is included in broiler diets and no more than 10% in layer diets. The addition of methionine and fat to these diets is a recommended practice in order to overcome the deficit in these nutrients. At this low level of usage, the cyanide content in dried forage does not constitute a limiting factor.

Dried cassava foliage meal for broilers

Low (less than 6%) levels of cassava foliage meal may be used, mainly as a natural skin pigmenter, with a very light negative effect on feed consumption and weight gain. When the inclusion of the foliage is higher than 6%, the growth rate and feed consumption are negatively affected. When a high level (more than 15%) of cassava foliage is compared with alfalfa meal, the performance results are negatively affected in both treatments, but a larger effect is observed for cassava foliage (**Table 35**).

Table 35. Effect of including high levels of cassava foliage meal or alfalfa meal for Leghorn broilers [1)].

Ingredients (%)				
Cassava foliage meal	15.0	--	20.0	--
Alfalfa meal	--	15.0	--	20.0
Corn	53.6	53.6	51.9	51.9
Soybean meal	19.9	19.9	16.6	16.6
Tuna fish meal	5.0	5.0	5.0	5.0
Meat and bone meal	5.0	5.0	5.0	5.0
Vitamins & minerals	1.5	1.5	1.5	1.5
Performance of broilers				
Weight at 3 weeks, g	191	212	186	203
Daily feed consumption, g	21.8	21.5	22.5	21.6
Feed conversion ratio	2.40	2.13	2.54	2.24

[1)] 1-21 day old broilers.

Source: *Ross and Enriquez, 1969.*

Table 36 also shows the results of diets with high levels (20%) of cassava foliage and the effect of methionine supplementation, since this aminoacid becomes limiting in this type of diets. The growth rate is negatively affected with high foliage content. However, up to 0.3% methionine addition improves the growth performance, although it does not reach the levels obtained by broilers consuming high energy diets.

Table 36. Effect of including a high level of cassava foliage meal and different levels of methionine in the feed of Leghorn broilers [1)].

Ingredients (%)		
Cassava foliage meal	--	20.0
Corn	66.5	51.9
Soybean meal	22.0	16.6
Tuna fish meal	5.0	5.0
Meat and bone meal	5.0	5.0
Vitamins & minerals	1.5	1.5
Methionine addition	Body weight at 21 days (grams)	
0	208	114
0.2 %	220	185
0.3 %	--	211
0.4 %	--	205
0.5 %	--	202
Methionine addition	Feed conversion rate	
0	2.10	2.73
0.2 %	1.99	2.32
0.3 %	--	2.18
0.4 %	--	2.35
0.5 %	--	2.18

[1)] 1-21 day old broilers.

***Source**: Ross and Enriquez, 1969.*

Dried cassava foliage meal for layers

Little information is available in performance of lagers fed cassava foliage diets, except in relation to its pigmenting effect on egg yolk. **Table 37** shows the effect of low levels (2.5 and 5.0%) of cassava foliage meal when added to white corn diets in comparison with yellow corn diets. There is a linear response to higher levels of cassava foliage, although the pigmenting effect of yellow corn is still superior. Cassava foliage meal at levels around 8% show a pigmenting effect similar to yellow corn, without affecting the performance of layers.

PERFORMANCE RESULTS WITH DRIED CASSAVA FOLIAGE IN SWINE FEEDING

Once again, since pigs are monogastric animals, the inclusion of cassava foliage does not have an important role in commercial feeding programs, especially for high energy demanding growing-fattening pigs. Gestating and lactating females provide a larger space for the inclusion of a higher percentage of cassava foliage, considering the need for crude fiber during these stages.

The high fiber content, low energy and poor palatability of dried cassava foliage are the main limiting factors for its inclusion in swine diets.

As a general recommendation it is suggested that no more than 8% of cassava foliage meal may be included in the diets of growing-finishing pigs, no more than 15% in gestating females, and no more than 10% in lactating females. At this low level of usage,

the cyanide content in dried foliage (200-500 ppm) does not constitute a potential danger of cyanide poisoning in pigs. Methionine and fat supplementation is a recommended practice whenever cassava foliage is included.

Table 37. Effect of including low levels of cassava foliage meal on egg yolk pigmentation of Leghorn layers.

Ingredients (%)				
Cassava foliage meal	--	2.5	5.0	--
White corn	68.5	66.0	63.5	--
Yellow corn	--	--	--	68.5
Wheat bran	2.5	19.9	16.6	16.6
Dextrose	0.5	0.5	0.5	0.5
Fish meal	2.5	2.5	2.5	2.5
Peanut meal	5.0	5.0	5.0	5.0
Soybean meal	13.0	13.0	13.0	13.0
Vitamins & minerals	8.0	8.0	8.0	8.0
Egg yolk pigmentation				
Grade on Roche scale	1.0	4.9	5.4	9.5

Source: *Agudu, 1972.*

Dried cassava foliage meal for growing-finishing pigs

Some of the early studies (**Table 38** and **39**) showed the effect of including more than 10% of dried cassava foliage in growing-finishing feeding programs. In every case there was a reduction in feed consumption and growth rate of pigs, even though the non-cassava foliage diets still did not have the needed energy concentration for modern genetic pig breeds. In the high demanding energy diets of modern lines, metabolizable energy and methionine supplementation are key factors to partially counteract the poor production performance with high cassava forage diets. These nutrient adjustments may be obtained if the dried cassava foliage is included at levels not larger than 6-8%.

Table 38. Effect of including high levels of dried cassava foliage meal in Landrace x Yorkshire growing pigs [1)].

Ingredients (%)				
Cassava foliage meal	--	10.0	20.0	20.0
Corn	74.40	66.85	59.85	59.65
Fish meal	8.0	7.0	7.0	7.0
Meat and bone meal	7.0	7.0	5.0	5.0
Soybean meal	7.95	6.50	5.50	5.50
DL-methionine	--	--	--	0.20
Vitamins & minerals	2.65	2.65	2.65	2.65
Performance of pigs				
Daily weight gain, kg	0.35	0.31	0.29	0.32
Daily feed consumption, kg	1.21	1.10	1.08	1.13
Feed conversion ratio	3.42	3.52	3.79	3.50

[1)] Growing pigs with initial weight of 13.6 kg, consuming isoproteic (18%) diets.

Source: *Choo and Hutagalung, 1972.*

Table 39. Effect of including high levels of dried cassava foliage meal in Landrace x Yorkshire growing pigs [1)].

Ingredients (%)					
Cassava foliage meal	--	20.0	20.0	20.0	20.0
Corn	77.6	57.1	52.1	54.1	51.9
Soybean meal	14.8	10.3	10.3	10.3	10.3
Fish meal	2.5	2.5	2.5	2.5	2.5
Meat and bone meal	2.5	2.5	2.5	2.5	2.5
Molasses	--	5.0	10.0	5.0	10.0
Palm oil	--	--	--	3.0	--
DL-methionine	--	--	--	--	0.20
Vitamins & minerals	2.6	2.6	2.6	2.6	2.6
Performance of pigs					
Daily weight gain, kg	0.53	0.43	0.46	0.44	0.50
Daily feed consumption, kg	1.90	1.66	1.71	1.68	1.84
Feed conversion rate	3.60	3.90	3.74	3.80	3.68

[1)] Growing pigs with initial weight of 31 kg, consuming isoproteic (18%) diets.
Source: *Choo and Hutagalung, 1972.*

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CHAPTER 27

RECENT DEVELOPMENTS WITH DRIED CASSAVA ROOTS AND FOLIAGE MEAL FOR POULTRY AND SWINE [1]

Julián Buitrago A., DVM, PhD. [2]

Although cassava meal can be combined with several ingredients in order to obtain balanced diets, the fullfat soybean (FFSB) has become a strategic product considering its nutritional benefits which somehow complements some of the cassava limitations. FFSB refer to the heat processed soybeans, through extrusion or toasting processes, which will guarantee the needed temperature to eliminate the antinutritional factors (trypsin inhibitors, hemaglutinins and lipoxygenase) present in raw soybeans.

The inclusion of cassava meal and FFSB as the main ingredients in diets for poultry and swine, simplifies the feeding programs in most of their productive stages, where there is a high need for metabolizable energy, essential aminoacids, lecithin and fatty acids. Cassava is rich in starches and energy, but poor in essential aminoacids and fatty acids. On the other hand, FFSB are poor in starches, but rich in essential protein, lecithin and essential fatty acids.

As **Table 1** indicates, the low concentration of some essential nutrients observed in cassava root meal (CRM) can be satisfactorily compensated for by their high concentrations in FFSB.

Table 1. Main nutritional differences between cassava root meal and fullfat soybeans.

Nutrients	UNIT	CRM	FFSB
Protein	%	2.8	38.0
Fat	%	1.2	19
Starch	%	70	9
ME, poultry	Mcal/kg	3.1 - 3.2	3.6 - 3.8
ME, swine	Mcal/kg	3.2 - 3.4	3.7 - 3.8
Linoleic acid	%	0.2	8.9
Fiber	%	2.6	4.9
Ash	%	3.2	5.2
Methionine	%	0.03	0.51
Cystine	%	0.02	0.60
Lysine	%	0.05	2.31
Threonine	%	0.05	1.43
Thryptophane	%	0.02	0.52
Lecithin	%	0.1	2.1

Source: *Buitrago, 1990.*

[1] For color photos see pages 794-795.

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In consideration to the previous observations, the following sections of this document will present various animal feeding programs for broilers, layers and pigs, based on different combinations of cassava root meal and fullfat soybeans (extruded or toasted FFSB).

BROILER FEEDING

Since the balanced feed for broilers is generally prepared in the form of a pelletized or crombelized product, the recommendations for the levels of cassava root meal that can be used may go as high as the total substitution of cereal grains in diets for starting and finishing broilers. The dusty feature of diets with high levels of cassava meal is totally overcome during the pelletization process, without the need of agglutinants or special additives. The high oil content of these diets, due to the inclusion of FFSB, is also an important factor to improve the pellet quality. Moreover, this type of diets allows the incorporation of maximum levels of cassava root meal (45-50%) as well as the needed quantity of cassava foliage meal (5-6%) in order to guarantee the proper pigmentation of broiler skins.

When the starting point is the mixture of cassava root meal, cassava foliage meal (CFM), FFSB and soybean meal (SBM), it is possible to formulate perfectly balanced diets for broilers, following the most recent NRC and AEC nutritional requirements, in which these three ingredients can represent more than 95% of the total feed, as illustrated in **Table 2. Table 3** provides more detailed information about the nutritional composition of the above mixtures.

Table 2. Broiler diets mainly based on cassava root meal, cassava foliage meal and fullfat soybeans.

Ingredients (%)	Starter (0-3 weeks)	Finisher (3-6 weeks)	Finisher (6-8 weeks)
Cassava root meal	41.05	44.70	50.50
Cassava foliage meal	--	6.0	6.0
Fullfat soybeans	44.50	44.74	40.80
Soybean meal	10.60	1.40	--
DL-methionine	0.25	0.16	0.10
Dicalcium phosphate	1.70	1.30	1.00
Calcium carbonate	1.20	1.00	0.90
Salt	0.30	0.30	0.30
Vitamins, minerals, additives	0.40	0.40	0.40

Table 3. Nutritional composition of broiler diets mainly based on cassava root meal, cassava foliage meal and fullfat soybeans.

Nutrients	Starter (0-3 weeks)	Finisher (3-6 weeks)	Finisher (6-8 weeks)
ME, Mcal / kg	3.20	3.20	3.20
Protein, %	23.0	20.0	18.0
Lysine, %	1.30	1.15	1.00
Methionine, %	0.55	0.43	0.34
Methionine + cystine	0.90	0.72	0.60
Threonine, %	0.85	0.78	0.69
Tryptophane, %	0.30	0.25	0.20
Fiber, %	4.3	5.0	4.8
Fat, %	8.8	8.9	8.3
Ash, %	7.2	6.6	6.1
Calcium, %	1.00	0.90	0.80
Available phosphorus, %	0.45	0.36	0.30
Linoleic acid, %	3.5	3.8	3.5

Performance results based on diets with low and medium levels of cassava meal

Even though the results obtained with the total replacement of cereal grains by cassava meal in pelletized diets have demonstrated that this criterion may become a viable practice in commercial feeding programs for broilers, it is possible that in many occasions, it is more convenient to use a partial substitution of the traditional cereal grains. This last modality is even a must when the diets are prepared in meal or flour presentation, considering the dusty characteristics of the cassava root meal. Nevertheless, pelletization or extrusion is always a very useful practice whenever cassava root meal or other dusty products are used in a considerable percentage of the diet.

Tables 4 and **5** illustrate the composition of the diets with intermediate levels of cassava meal plus FFSB, in which the objective was the substitution of about 40-50% of the corn or sorghum used in pelletized diets for the starting (0-21 days) and finishing (21-42 days) phases.

Based on previous laboratory trials conducted with a small number of animals, the above diets were then tested with a larger number of chickens on commercial farms in two locations: diets from **Table 4** were tested under mild environmental conditions in the Cauca Valley of Colombia (24^{o}C, 78% humidity, 1050 masl), and diets from **Table 5** were tested under a warmer environment (32^{o}C, 86% humidity, 40 masl) near the north coast of Colombia (Cereté, Córdoba). 15,350 birds were used in the first trial and 72,400 birds were used in the second trial. In both cases, the cassava diets were compared with corn-soybean meal commercial diets with similar nutrient composition.

Table 4. Composition of broiler diets with intermediate levels of cassava meal and fullfat soybeans [1)].

Ingredients (%)	Starting	Finishing
Corn	25.34	30.79
Cassava roots meal	25.0	25.0
FFSB (toasted)	31.4	33.8
Soybean meal	12.1	4.8
Chicken viscera meal	3.00	3.00
Dicalcium phosphate	1.30	1.00
Calcium carbonate	1.00	0.90
DL-methionine	0.23	0.10
Salt	0.35	0.30
Vitamins and minerals	0.12	0.10
Anticoccidial	0.05	0.10
Fungicide	0.10	0.10
Nutrient composition		
ME., Mcal/kg	3.10	3.20
Protein, %	22.0	17.0
Methionine, %	0.56	0.40
Met + Cystine, %	0.90	0.72
Lysine, %	1.24	1.10
Threonine, %	0.80	0.75
Linoleic acid, %	3.25	3.48
Calcium, %	0.90	0.82
Available phosphorus, %	0.42	0.39

[1)] Commercial Farm El Recreo–Carioca. Buga, Colombia.

Source: *Buitrago et al., 2002.*

The results obtained with respect to the performance of broilers are shown in **Tables 6** and **7.** In general, it can be concluded that broilers consuming diets with a substitution of 50% of corn or sorghum by cassava root meal had the same (or better) performance than those that consumed the conventional diets with cereal grains. In terms of weight increase, feed conversion ratio and carcass yield, there were no significant differences. Adverse effects, above the normal figures, were not observed in terms of mortality or morbidity as a result of the inclusion of cassava root meal as the main energy source plus FFSB as the main protein source. Differences in humidity of the litter used in the different poultry houses were not appreciable either.

Performance results based on diets with maximum levels of cassava root and cassava foliage meal

Experimental work conducted at CIAT compared a commercial pelletized broiler diet based on corn and soybean meal with pelletized diets totally based on cassava root and cassava foliage meal supplemented with FFSB. The comparison between solar dehydration and artificial dehydration of cassava roots was also included in the same study. A detailed description of the experimental diets as well as its nutritional composition for the starting (0-21 days) and finishing (21-42 days) phases is presented in **Tables 8** and **9.**

Table 5. Composition of broiler diets with intermediate levels of cassava meal and FFSB [1].

Ingredients (%)	Starting	Finishing
Cassava roots meal	20.0	25.0
FFSB (toasted)	32.0	34.0
Soybean meal	8.20	2.80
Fish meal	3.50	4.00
Palm oil	--	0.10
Dicalcium phosphate	0.90	0.70
Calcium carbonate	0.80	0.90
DL-methionine	0.27	0.22
Salt	0.25	0.25
Chline chloride	0.12	0.10
Vitamins and minerals	0.12	0.10
Anticoccidial	0.05	0.10
Fungicide	0.10	0.10
Nutrient composition		
ME., Mcal/kg	3.15	3.20
Protein, %	21.0	19.0
Methionine, %	0.58	0.51
Met + Cystine, %	0.88	0.77
Lysine, %	1.23	1.10
Threonine, %	0.60	0.59
Linoleic acid, %	3.08	3.10
Calcium, %	0.90	0.91
Available phosphorus, %	0.43	0.42

[1] Commercial Farms: Avités – Nutrilisto. Cereté, Colombia.

Source: *Buitrago et al., 2002.*

Table 6. Results on the performance of broilers with intermediate levels of cassava root meal in the diet [1].

	Control (corn – SBM)[2]	Cassava -FFSB[3]
Number of birds at starting	7.680	7.673
Number of birds at finishing	7.415	7.108
Number of days	42	42
Mortality, %	3.2	5.7
Final weight, g	1.976	1.942
Feed consumption, g	3.754	3.781
Conversion efficiency	1.90	1.94
European conversion efficiency	239	218

[1] El Recreo Farm. Buga, Cauca Valley, Colombia.

[2] Control commercial diet based on corn and soybean meal.

[3] Experimental diet based on cassava root meal and fullfat soybeans.

Source: *Buitrago et al., 2002.*

Table 7. Results on the performance of broilers with intermediate levels of cassava root meal in the diet [1].

	Control (sorghum-SBM)[2]	Cassava -FFSB[3]
Number of birds at starting	48.441	24.000
Number of birds at finishing	46.199	22.392
Number of days	42	42
Mortality, %	4.6	6.7
Final weight, g	1.934	1.915
Feed consumption, g	3.559	3.152
Feed conversion ratio	1.84	1.69
European conversion efficiency	239	218

[1] Avites Farm. Cereté, Cordoba, Colombia.
[2] Control commercial diet based on sorghum and soybean meal.
[3] Experimental diet based on cassava root meal and fullfat soybeans.
Source: *Buitrago et al., 2002.*

Performance results demonstrated the feasibility of preparing broiler feeding programs totally based on cassava root meal as the main energy source and limited levels of cassava foliage meal as a partial protein source, as long as FFSB is included to provide the deficit of energy, fatty acids and protein.

Table 10 shows the overall performance of broilers until 42 days when the trial was finished. All groups consuming cassava products and FFSB obtained similar or better weight gains and feed conversion ratios when compared to the control group fed with corn and soybean meal. The consumption of the balanced feed was not affected by the inclusion of high levels of cassava meal during the starting and finishing production phases.

In the treatments that included cassava root meal, the effect of artificial drying was superior to the sun drying procedure. Both steam and gas drying equipments were equally effective for the drying process. The high temperature obtained during the artificial drying facilitates the gelatinization of starches and the control of pathogenic germs. These two factors have probably an important influence on the superior performance of these groups when compared with the sun dried cassava group.

Although the diets with a high percentage of cassava meal and FFSB contain high potassium levels in their final composition, it was not observed to have an adverse effect on the chicken manure and humid litters. Humidity of the manure was analyzed at weekly intervals and no significant differences were observed. Additionally, the measure of the moisture content of the litter did not indicate differences among groups.

Through external measurements of the skin and by checking the chicken carcasses after sacrifice, pigmentation of legs, skin and internal fat was analyzed. The groups with diets based on just cassava roots showed a poor pigmentation, while the group with cassava roots and foliage showed a pigmentation grade similar to that of the control group fed with diets based on yellow corn. The visual appreciation on a scale from 1 (pale) to 5 (optimum pigmentation), gave both the control and the group fed with cassava roots plus foliage meal a grade of 4, while the other groups without cassava foliage obtained a grade of 2 on the pigmentation scale.

Table 8. Composition of broiler diets with maximum levels of cassava meal and FFSB in the starting phase.

Ingredients (%)	Control (corn-SBM)	CRM + FFSB [1)]			CRM+CFM + FFSB[2)]
		Solar drying	Artificial drying		
			A [3)]	B [4)]	
Corn	59.37	--	--	--	--
CRM	--	45.75	45.75	45.75	40.45
CFM	--	--	--	--	6.00
FFSB	12.8	30.0	30.0	30.0	30.0
Soybean meal	21.0	18.7	18.7	18.7	18.7
Palm oil	3.0	2.9	2.9	2.9	4.5
DL-methionine	0.16	0.29	0.29	0.29	0.29
L-lysine	0.07	--	--	--	--
Bone meal	1.70	1.90	1.90	1.90	1.90
Ca carbonate	1.50	--	--	--	--
Salt	0.30	0.30	0.30	0.30	0.30
Vitamin Premix	0.10	0.10	0.10	0.10	0.10
Nutrient composition					
ME., Mcal/kg	3.20	3.20	3.20	3.20	3.20
Protein, %	22.0	22.0	22.0	22.0	22.0
Methionine, %	0.59	0.59	0.59	0.59	0.59
Met + Cystine, %	0.90	0.90	0.90	0.90	0.90
Lysine, %	1.26	1.26	1.26	1.26	1.27
Linoleic acid, %	2.62	3.42	3.42	3.42	3.56
Ca, %	0.91	0.91	0.91	0.91	0.91
Available P, %	0.42	0.42	0.42	0.42	0.42

[1)]Cassava root meal + fullfat soybeans.
[2)]Cassava root meal + cassava foliage meal + fullfat soybeans.
[3)]Equipment with steam heating
[4)]Equipment with propane gas heating
Source: *Gil et al., 2000.*

LAYER FEEDING

Feeding programs for layers generally involve the use of diets in meal or flour presentation, which becomes an important limitation for the inclusion of high levels of cassava roots meal due to the dustiness of the final product. This situation is no longer a problem when low or medium levels of cassava root meal are included. Unless the possibility of using pelletized or crombelized diets is considered, it is difficult to incorporate levels higher than 25% of cassava root flour in layer feeding.

In relation to cassava foliage meal, it is also recommended that its use in diets should not exceed levels of 6% in order to minimize the negative effects on palatability or high HCN presence in the feed. When high quality foliage meal is included at levels between 5 and 6%, a satisfactory pigmentation of egg yolks is obtained, due to the presence of natural xanthophylls.

Table 11 illustrates an example of diets for replacement layer chickens and laying hens based on maximum levels of cassava root meal combined with FFSB and 6% foliage meal, in which these ingredients can represent up to 85% of the total feed. The corresponding nutritional components are shown in **Table 12. Tables 13** and **14** show similar examples in which cassava root meal has been restricted to levels not higher than 25% of the chicken and layer diets.

Table 9. Composition of broiler diets with maximum levels of cassava meal and FFSB in the finishing phase.

		CRM + FFSB [1]			
	Control		Artificial drying		CRM+CFM
Ingredients (%)	(corn-SBM)	Solar drying	A [3]	B [4]	+ FFSB [2]
Corn	66.85	--	--	--	--
CRM	--	49.8	49.8	49.8	46.1
CFM	--	--	--	--	6.00
FFSB	6.1	41.6	41.6	41.6	45.1
Soybean meal	20.7	5.2	5.2	5.2	--
DL-methionine	0.13	0.23	0.23	0.23	0.23
Lysine	0.19	--	--	--	--
Bone meal	1.60	1.90	1.90	1.90	1.90
Ca carbonate	1.10	--	--	--	--
Salt	0.30	0.30	0.30	0.30	0.30
Vitamin Premix	0.10	0.10	0.10	0.10	0.10
Nutrient composition					
ME., Mcal/kg	3.20	3.20	3.20	3.20	3.20
Protein, %	20.0	20.0	20.0	20.0	20.0
Methionine, %	0.49	0.49	0.49	0.49	0.49
Met + Cystine, %	0.78	0.78	0.78	0.78	0.78
Lysine, %	1.12	1.12	1.12	1.12	1.12
Linoleic acid, %	2.20	3.60	3.60	3.60	3.85
Ca, %	0.90	0.90	0.90	0.90	0.90
Available P, %	0.40	0.40	0.40	0.40	0.40

[1] Cassava root meal + fullfat soybeans.
[2] Cassava root meal + cassava foliage meal + fullfat soybeans.
[3] Equipment with steam heating
[4] Equipment with propane gas heating
Source: *Gil et al., 2000.*

Table 10. Results on the performance of broilers with maximum levels of cassava root meal and FFSB in the diet during the starting and finishing phases.

Ingredients (%)	Control (corn-SBM)	CRM + FFSB[1)]			CRM+CFM + FFSB[2)]
		Solar drying	Artificial drying		
			A[3)]	B[4)]	
Initial weight, g	39.8	39,5	39.4	39.5	39.7
Final weight, g	2,139	2,279	2,237	2,387	2,113
Feed consumption	4.73	4.88	4.65	4.68	4.72
Feed conversion rate	2.21	2.14	2.08	1.96	2.24

[1)] Cassava root meal + fullfat soybeans
[2)] Cassava root meal + cassava foliage meal + fullfat soybeans
[3)] Equipment with steam heating
[4)] Equipment with propane gas heating
Source: *Gil et al., 2001*.

Table 11. Example of layer diets with maximum levels of cassava root meal, fullfat soybean and cassava foliage meal.

Ingredients (%)	Replacement chickens		Laying hens	
	0-6 weeks	7-15 weeks	Phase 1	Phase 2
Cassava root meal	59.3	61.4	41.6	51.9
FFSB	9.6	9.2	38.9	28.0
Cassava foliage meal	--	6.0	6.0	6.0
Soybean meal	26.9	19.6	1.9	3.6
Calcium phosphate	1.4	1.2	1.2	1.2
Calcium carbonate	1.9	1.8	9.5	8.4
DL-methionine	0.21	0.10	0.23	0.23
Salt	0.30	0.30	0.30	0.30
Vitamin & minerals	0.40	0.40	0.40	0.40

Table 12. Nutritional composition of layer diets with maximum levels of cassava root meal, fullfat soybeans and cassava foliage meal [1].

	Replacement chickens		Laying hens	
Ingredients (%)	0-6 weeks	7-15 weeks	Phase 1	Phase 2
Metabolizable energy, Mcal/kg	2.80	2.75	2.90	2.80
Protein, %	18.0	15.5	18.0	15.0
Lysine	0.98	0.68	0.86	0.75
Methionine	0.42	0.30	0.38	0.36
Met + Cystine	0.72	0.54	0.73	0.64
Threonine	0.65	0.60	0.66	0.50
Calcium	0.90	1.10	4.00	3.60
Available phosphorus	0.38	0.35	0.32	0.32
Fiber	3.8	4.6	4.6	4.4
Fat	2.0	2.9	7.8	6.0
Linoleic acid	1.0	1.0	2.5	2.4
Ash	6.6	7.2	14.2	13.0

[1] Nutrient requirements based on NRC and AEC recommendations.

Table 13. Example of layer diets with medium levels of cassava root meal, fullfat soybeans and cassava foliage meal.

	Replacement chickens		Laying hens	
Ingredients (%)	0-6 weeks	7-15 weeks	Phase 1	Phase 2
Corn	39.0	42.9	19.7	31.0
Cassava root meal	25.0	25.0	25.0	25.0
FFSB	10.0	9.84	34.6	19.1
Cassava foliage meal	--	6.0	6.0	6.0
Soybean meal	21.6	12.2	2.8	7.2
Calcium phosphate	1.3	1.2	1.1	1.1
Calcium carbonate	2.20	2.10	9.9	9.7
DL-methionine	0.14	0.06	0.20	0.17
Salt	0.30	0.30	0.30	0.30
Vitamin & minerals	0.40	0.40	0.40	0.40

Performance results based on diets with medium and low levels of cassava meal

Field experiments have been conducted in one of the main poultry regions of Colombia (Cauca Valley). In all feeding trails the diets were prepared in meal or flour form and the level of replacement of corn was not more than 50%.

Tables 15, **17, 19** and **21** show the composition of the diets used in several experiments conducted in commercial layer farms, during different laying periods. Cassava root meal was included at levels from 10 to 20% of the total diet. FFSB, either extruded or toasted, was used in all cases at levels not higher than 20%.

Table 14. Nutritional composition of layer diets with medium levels of cassava root meal, fullfat soybeans and cassava foliage meal [1)].

	Replacement chickens		Laying hens	
	0-6 weeks	7-15 weeks	Phase 1	Phase 2
Metabolizable energy, Mcal/kg	2.80	2.75	2.90	2.80
Protein, %	18.0	15.5	18.0	15.0
Lisine, %	0.98	0.68	0.86	0.75
Methionine	0.42	0.30	0.38	0.36
Met + Cystine	0.72	0.54	0.73	0.64
Threonine	0.65	0.60	0.66	0.50
Calcium	0.90	1.10	4.00	3.60
Available phosphorus	0.38	0.35	0.32	0.32
Fiber	3.8	4.6	4.6	4.4
Fat	2.0	2.9	7.8	6.0
Linoleic acid	1.0	1.0	2.5	2.4
Ash	6.6	7.2	14.2	13.0

[1)] Nutrient requirements based on NRC and AEC recommendations.

Table 15. Diets for commercial layers with 10% cassava root meal and fullfat soybeans.

Ingredients (%)	Control (corn)	10% Cassava root meal
Corn	57.8	45.3
Cassava root meal	--	10.0
FFSB (toasted)	5.3	9.1
Soybean meal	16.2	15.0
Fish meal (65 % protein)	5.0	5.0
Wheat bran	3.5	3.5
DL-methionine	0.18	0.20
Calcium carbonate	9.71	9.64
Calcium phosphate	0.95	0.91
Salt	0.30	0.30
Vitamins and minerals	0.60	0.60
Nutrient composition		
ME, Mcal/kg	2.75	2.75
Protein, %	17.5	17.5
Methionine, %	0.44	0.44
Met + Cystein, %	0.75	0.75
Lysine, %	0.91	0.91
Calcium, %	3.90	3.90
Available phosphorus, %	0.45	0.45
Linoleic acid, %	1.36	1.39

Table 16. Performance of commercial layers fed with 10% cassava root meal and fullfat soybeans[1)].

	Control (corn)	10% Cassava root meal
Daily feed consumption, g	102.6	103.2
Laying, %	89.2	89.5
Feed conversion (per dozen eggs)	1.4	1.4

[1)] 48-55 week laying period
La Esperanza Poultry Farm. Buga, Valle. 1,010 masl. 26°C.
Source: *Gutierrez and Martinez, 1998.*

Table 17. Diets for commercial layers with 15% cassava root meal and fullfat soybeans.

Ingredients (%)	Control (corn)	15% Cassava root meal
Corn	41.1	34.1
Cassava root meal	--	15.0
Fullfat soybeans (extruded)	20.0	20.00
Soybean meal	8.1	11.60
Rice polishings	10.0	--
Wheat bran	9.1	7.60
DL-methionine	0.18	0.19
Calcium carbonate	9.60	9.30
Calcinated bone meal	1.30	1.50
Salt	0.35	0.35
Vitamins and minerals	0.30	0.30
Nutrient Composition		
ME, Mcal/kg	2.75	2.75
Protein, %	17.0	17.0
Methionine, %	0.45	0.45
Met + Cystine, %	0.70	0.70
Lysine, %	0.85	0.85
Calcium, %	3.90	3.90
Available phosphorus, %	0.42	0.42
Linoleic acid, %	1.74	1.37

Results in productivity of layers fed the experimental diets already described are presented in **Tables 16, 18, 20, 22** and **23.**

No important differences were observed in the production parameters of all experiments. Laying percentage and feed conversion was similar in diets with no cassava root meal compared to diets with 10, 15 and 20% cassava root meal. A slight reduction in egg laying percentage and feed conversion was observed in brown layers fed with 10 or 20% cassava root meal (**Table 23**).

Table 18. Performance of commercial layers fed with 15% cassava root and fullfat soybeans [1].

	Control (corn)	15% Cassava root meal
Layers, No.	15,000	5,000
Daily feed consumption, g	114.0	115.0
Laying, %	78.3	79.0
Feed conversion (dozen eggs)	1.37	1.37

[1] 55-61 week laying period
Santa Anita Poultry Farm. Pradera, Valle. 1.010 masl. 26°C.
American Soybean Association (ASA), 2000.

Source: *Buitrago et al., 2002.*

Table 19. Diets for commercial layers with 20% cassava root meal and fullfat soybeans.

Ingredients (%)	Control (corn)	20% Cassava root meal
Corn	20.0	--
Sorghum	30.6	36.2
Cassava root meal	--	20.0
FFSB (toasted)	15.0	15.0
Soybean meal	12.3	16.5
Wheat bran	10.3	0.20
DL-methionine	0.23	0.23
Calcium carbonate	9.20	9.30
Calcium phosphate	1.40	1.60
Salt	0.35	0.35
Vitamins and minerals	0.60	0.60
Nutrient composition		
ME, Mcal/kg	2.70	2.70
Protein, %	17.0	17.0
Methionine, %	0.45	0.45
Met + Cystein, %	0.70	0.70
Lysine, %	0.81	0.81
Calcium, %	3.90	3.90
Available phosphorus, %	0.42	0.42
Linoleic acid, %	1.54	1.25

Table 20. Performance of commercial layers fed with 20% cassava root meal and fullfat soybeans [1].

	Control corn	20% Cassava root meal
Daily feed consumption, g	111.6	111.1
Laying, %	92.4	91.0
Feed conversion (per dozen eggs)	1.50	1.46

[1] 39-46 week laying period
Avícola Montegrande Poultry Farm. Tuluá, Valle. 1,025 masl. 25°C.

Source: *Gutierrez and Martínez, 1998.*

Table 21. Diets for commercial white and brown layers with 10% and 20% cassava root meal and fullfat soybeans.

Ingredients (%)	Control (corn)	10% Cassava root meal	20% Cassava root meal
Corn	41.1	34.1	23.0
Cassava root meal	--	10.0	20.0
FFSB (extruded)	20.0	20.0	20.0
Soybean meal	8.1	10.4	11.8
Rice polishings	10.0	10.0	10.0
Wheat bran	9.1	4.3	3.6
DL-methionine	0.18	0.19	0.21
Calcium carbonate	9.60	9.50	9.40
Calcinated phosphate	1.30	1.40	1.40
Salt	0.35	0.35	0.35
Vitamins and minerals	0.30	0.30	0.30
Nutrient composition			
ME, Mcal/kg	2.70	2.70	2.70
Protein, %	17.0	17.0	17.0
Methionine, %	0.45	0.45	0.45
Met + Cystein, %	0.70	0.70	0.70
Lysine, %	0.85	0.85	0.85
Calcium, %	3.90	3.90	3.90
Available phosphorus, %	0.42	0.42	0.42
Linoleic acid, %	1.74	1.49	1.37

Table 22. Performance of commercial white layers fed with 10% cassava root meal and fullfat soybeans [1)].

	Control (corn)	10% Cassava root meal
Layers, No.	10,464	8,976
Daily feed consumption, g	107.5	105.5
Laying, %	64.1	63.0
Feed conversion (per dozen eggs)	2.01	2.01

[1)] 78-88 week laying period. Hy-line layers.
Avicauca Poultry Farm. Jamundí, Valle. 1,005 masl. 25°C.
American Soybean Association (ASA), 1999.

Source: *Buitrago et al., 2002.*

Table 23. Performance of commercial brown layers fed with 10% and 20% cassava root meal and fullfat soybeans [1)].

	Control (corn)	10% Cassava root meal	20% Cassava root meal
Layers, No.	3,840	10,956	5,160
Daily feed consumption, g	115.1	115.8	114.8
Laying, %	69.3	65.7	65.1
Feed conversion (per dozen eggs)	2.00	2.12	2.11

[1)] 78-88 week laying period. Lohmann Brown layers.
Avicauca Poultry Farm. Jamundí, Valle. 1,005 masl. 25°C.
American Soybean Association (ASA), 1999.

Source: *Buitrago et al., 2002.*

SWINE FEEDING

Nutritional considerations already analyzed in poultry feeding based on cassava and FFSB have a close similarity with other monogastric animals, mainly swine. Cassava root meal and cassava foliage meal can partially or totally replace the conventional cereal grains in commercial diets. FFSB also provide key nutrients which will complement the nutritional weaknesses of cassava.

Table 24. Swine diets totally based on cassava root meal, cassava foliage meal and fullfat soybeans.

Ingredientes (%)	Starting	Growing	Final	Gestation	Lactation
Cassava root meal	45.2	50.5	53.4	57.1	51.7
Cassava foliage meal	--	4.0	8.0	8.0	8.0
Fullfat soybean	45.8	42.8	33.8	29.5	35.2
Soybean meal	6.0	--	--	--	--
Vegetable oil	--	0.4	2.8	3.0	2.8
Methionine	0.06	0.05	0.03	--	0.04
Dicalcium phosphate	1.2	0.8	0.5	1.1	1.0
Calcium carbonate	1.2	0.9	0.9	0.7	0.7
Salt	0.35	0.35	0.35	0.35	0.35
Vitamins & minerals	0.20	0.20	0.20	0.20	0.20
Nutrient composition					
ME, Mcal/kg	3.35	3.35	3.35	3.32	3.35
Protein, %	21.00	18.00	15.50	14.00	16.00
Lysine, %	1.20	0.95	0.75	0.58	0.95
Met + Cysteine, %	0.65	0.54	0.44	0.37	0.48
Calcium, %	0.90	0.90	0.88	0.90	0.86
Av. phosphorus, %	0.40	0.32	0.25	0.35	0.35

When cassava root flour is included at levels above 20%, the pelletization or extrudization processes are always recommended, especially for starting piglet diets. In growing-finishing pigs and breeding animals, pelletization is also recommended, although the addition of molasses, fat or FFSB can alleviate the dustiness of high cassava meal diets.

As in broiler and layer feeding, it is possible to formulate balanced diets for the different production stages in pigs, based on the mixture of cassava roots and cassava foliage meal, FFSB and soybean meal, in which these ingredients can represent more than 95% of the total feed, as illustrated in **Table 24.**

In recent studies, the inclusion of high levels of cassava root meal has been successfully proven in finishing diets where FFSB has been also included. The total replacement of cereal grains by cassava root meal is possible once the nutritional adjustments are introduced (**Tables 25** and **26**).

Table 25. High levels of cassava root meal and fullfat soybeans in diets for growing-finishing pigs.

	Control diet		Cassava root meal + FFSB	
Ingredients (%)	Growing	Finishing	Growing	Finishing
Corn	36.70	33.80	--	--
Cassava root meal	--	--	44.93	48.10
Fullfat soybean	20.00	18.60	20.00	20.00
Sorghum	16.00	16.00	--	--
Fish meal	--	0.50	--	--
Corn bran	8.00	12.00	--	--
Soybean meal	7.60	3.40	16.71	10.90
Wheat bran	8.00	12.00	12.00	15.00
Vegetable oil	--	--	3.70	3.30
Salt	0.39	0.39	0.39	0.39
Vitamin & minerals	3.31	3.31	2.27	2.31
Main nutrients				
ME, Mcal/kg	3.31	3.32	3.36	3.34
Protein, %	18.3	17.3	16.3	16.3

Table 26. Performance of finishing pigs with high inclusion of cassava root meal and fullfat soybean diets[1)].

	Control diet	Cassava root meal + FFSB
Initial weight, kg	48.10	49.29
Final weight, kg	96.00	96.41
Daily weight gain, kg	0.75	0.74
Daily consumption, kg	2.22	2.12
Feed conversión ratio	2.96	2.89

[1)] Granjas Paraíso – CLAYUCA – Nutribal. Palmira, Valle. 2002.

Source: *Buitrago et al., 2001.*

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CHAPTER 28

USE OF CASSAVA FOR SMALL-SCALE ETHANOL PRODUCTION WITH VALUE-ADDED BY-PRODUCTS [1]

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INTRODUCTION

Bioenergy, and biofuels in particular, have become major issues on the global agricultural research and development agenda, because of the enormous potential to help overcome the problems associated with the use of fossil fuels (decline in reserves, increased use, increased prices, increased emissions of greenhouse gases and direct impacts on climate change). As a consequence, there is growing concern and urgent need of governments, especially in less developed countries, to provide farmers with job opportunities that could help them to improve their incomes and promote sustainable economic development.

The technology that is currently available for the production of bioethanol has partially filled the expectations of reducing environmental problems caused by increased use of fossil fuels. The same cannot be said for two other key components that the world is demanding in the different production systems, i.e. economic sustainability and social development. The vast majority of ethanol production systems in the world have adopted a model based on monocultures (sugarcane and maize) with serious environmental problems, in terms of loss of biodiversity, excessive water use and generation of large amounts of waste water with high pollution potential. In addition, these systems require high investment for its establishment, a factor that prevents poor rural communities from participating and benefiting from these technologies. On the contrary, in many developing countries, the farmers are not only excluded from the bioethanol revolution but also end up affected by increases in prices of food commodities, reduced food security and increased poverty levels.

One of the main reasons for giving priority to the generation of bioenergy and the use of biofuels on the global agricultural development agenda is the possibility that these technologies could become strategies for reducing poverty and overcoming social inequalities that exist in many underdeveloped and developing countries. Some estimates suggest that there are more than 2 billion people worldwide who lack any access to modern

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[1] For color photos see pages 796-801.

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energy sources (UNDP, 2004). That is why it is imperative to design and implement technology platforms for the production, utilization and marketing of biofuels, with potential to help rural communities with few resources to minimize their dependence on fossil energy sources, and to achieve a more equitable distribution of the benefits across the entire agro-productive chain of the biofuels.

Rural Social Biorefineries - RUSBI: an approach for the production of biofuels on a small-scale

CIAT and CLAYUCA have been implementing since 2006 a research and development project with the aim of establishing a technology platform for producing hydrated bioethanol, at the level of small rural communities, using cassava, sweet potato and sweet sorghum as the feedstock crops.

This initiative, which has been named Rural Social Biorefineries – RUSBI – seeks to promote rural development in poor rural communities, located in marginal regions of Latin America and the Caribbean region, The RUSBI approach is not a technological package designed for biofuel production in large-scale commercial enterprises. On the contrary, RUSBI is an approach for small-scale production and local uses of biofuel, as a strategy to promote agricultural and economic development of billions of farmers around the world, living in marginal areas, facing a lack of resources, especially energy. RUSBI is meant to address the needs of these people and become an alternative model for promoting more inclusive, equitable bioenergy development efforts. The production of this biofuel is not considered the final product, as is the case of the commercial large-scale operations. In the RUSBI approach, the biofuel becomes an intermediate objective that allows farmer groups to have access to electric energy, and to use this new energy security status for implementing other agro-industrial transformation processes, adding value to their agricultural products and creating new employment and income opportunities, that help them to reduce their levels of poverty and improve their standards of living. The RUSBI approach focuses on developing an alternative approach for biofuel production that overcomes the social inequalities that characterize the modern, large-scale, commercial biofuel operations that are booming around the world, characterized by the limited participation of the farmers in the distribution of the benefits, acting merely as providers of raw material for the distilleries (Ospina *et al.*, 2009).

The RUSBI approach for biofuel production

The RUSBI approach for the production of biofuels includes five technological components that integrate modern concepts of agricultural management, process engineering and effluent management. (**Figure 1**).The end objective of the RUSBI approach is to promote agricultural development, food safety and energy self-sufficiency with small-scale farmer groups and rural communities, living in isolated, marginal areas. The scale of the rural social biorefinery is small to facilitate the participation of poor farmer groups: the capacity of the ethanol distillery is 20-25 liters per hour; groups of 10-20 farmer families could produce enough cassava, sweet potato and sweet sorghum to run the plant, and the total cost of the investment for a rural community is around 100,000 US$.

The different types of equipment included in a RUSBI, shown in **Figure 2,** are as follows: a) a pilot plant to produce hydrated ethanol (96%) with capacity to produce 20 liters per hour; b) a drying plant and a refining unit to produce cassava and sweet potato flour, and a milling section to produce sweet sorghum juice; and c) a plant for treating the effluents. The biorefinery equipment also includes a stationary engine to generate bioelectricity and a cooking stove. Both use the hydrated ethanol as fuel. The process for production of the hydrated ethanol in the RUSBI approach is shown in **Figure 3**.

Bioethanol production process

In the process of producing bio-ethanol using cassava as the feedstock, the cassava roots are processed first into flour and then converted into a slurry or liquid biomass, by adding water. At this stage, incubation conditions of the medium must be adjusted (pH and temperature) to continue with the following steps: hydrolysis and fermentation. This step could also be conducted with fresh cassava roots, which become a grated pulp with very fine particle size to facilitate the later stages of hydrolysis and fermentation. When using fresh cassava roots, less water is required for the process because it uses the water contained in the cassava roots, but the liquid obtained after fermentation must be filtered, as it has a higher fiber content. Additionally, when using cassava flour instead of fresh roots, the drying process allows for two products, cassava peels and fibers that can be sold for use in animal feed, helping to reduce the additional cost of energy required to convert the roots into flour.

Hydrolysis is one of the most important phases of the process, allowing the conversion of starch into fermentable sugars, which are then metabolized and assimilated by yeast during fermentation, producing bioethanol as a result. The enzymatic hydrolysis or saccharification is catalyzed by enzymes whose function is to break down large starch molecules to produce units of glucose. Starch-based glucose syrups are produced by the liquefaction and saccharification of the starch.

In the experience of CIAT-CLAYUCA with the RUSBI methodology to produce ethanol, a simultaneous hydrolysis and fermentation method has been used, to reduce processing time, power consumption and installation costs, since it does not require the installation of a heating system for production of the fermentation substrate.

To separate the ethanol from the fermented mash (end product of the HFS), a distillation stage is required, where ethanol is evaporated at 78°C. Ethanol vapors are captured and condensed, yielding ethanol with 96% purity and a liquid effluent called “vinasses”.

Finally, hydrated ethanol fuel has been evaluated on different equipment adapted for use as cooking stoves, electric power plants, motors and other devices for local use in rural communities (**Figure 4**). Validated uses of hydrated ethanol produced from cassava, could help rural communities to have access to electricity, enabling them to establish processing and adding value to agricultural products, and thus link to markets in which they could earn higher incomes and improve their food safety and quality of life.

Bioethanol production trials

Preliminary results obtained in CLAYCA-CIAT on the evaluation of cassava varieties for the production of bioethanol indicated that there is an enormous potential to exploit the genetic diversity of cassava and improve the process of transformation of biomass into ethanol. Considering the average value of starch found in the analyzed varieties, it was possible to estimate a theoretical value of 220 liters/t of fresh roots, and determine an experimental value of 118 liters/t of fresh roots, for the conversion of biomass into ethanol, which means that the real transformation efficiency is only 54% of the theoretical potential (**Table 1**) (Arriaga, 2008).

Table 1. Comparison of cassava varieties in the production of ethanol.

Variety	Production (t/ha)	Starch (%)	Theoretical conversion (L/t)	Real conversion (L/t)	Efficiency (%)	Production of ethanol (L/ha)
CM 4574-7	25	32.3	230.6	118.0	51	2950
CM6438-14	26	33.3	237.8	129.8	55	3374
MTAI-8	29	31.6	225.6	129.1	57	3743
Verónica	29	29.0	207.1	99.9	48	2897
Gines	27	27.9	199.2	114.7	58	3096
Average	27 ± 1.8	31 ± 2.3	220 ± 16.3	118 ± 12.2	54 ± 4.2	3212 ± 350

More recent work carried out by CLAYUCA-CIAT, has focused on optimizing the enzymatic hydrolysis of the starch present in cassava (Cajamarca, 2009), and the estimation of the efficiency in the production of bioethanol from cassava flour, at pilot scale, by calculating the mass and energy balances in the process (Martinez, 2009). Some of the tests with cassava flour in the pilot plant for production of hydrated ethanol are presented in **Table 2**.

According to the results in **Table 2**, the best treatment was the trial # 3. The yield was 372.5 liters of ethanol per ton of flour, and 106.4 liters per ton of fresh roots, values slightly lower than those reported in the literature (Vinh, 2003; Atthasampunna *et al.*, 1990).

It is also noted that for the efficiency of the process, a relatively low value (61%) was obtained, estimated in accordance with the actual production of ethanol, compared with the theoretical conversion. This implies the presence of pollutants, especially in the fermentation stage, which reduce or limit the fermentative glycolysis of the ethanol.

Table 2. Results of three tests for production of hydrated bio-ethanol from cassava flour at the CLAYUCA-CIAT pilot plant.

	Trial #1	*Trial #2*	*Trial #3*
Raw material			
Refined flour (kg)	75	86	120
Enzymess (*Stargen*) (kg)	0.375	0.428	0.600
Yeasts (*Ethanol red*) (kg)	0.250	0.286	0.400
Urea (kg)	0.175	0.200	0.300
Water (kg)	400	400	400
Products obtained			
Hydrated Ethanol 96% v/v (L)	21.8	27.3	44.7
Quantitative analysis			
Total Production total (L)	21.8	27.3	44.7
Yield (L ETOH/ton flour)	290.7	317.4	372.5
Yield (L ETOH/ton roots)[b]	83.1	90.7	106.4
Yield (L ETOH/ha)[c]	2076.4	2267.4	2660.0
Efficiency of production[d]	48%	52%	61%
Ratio vinasses/ethanol (v/v)	25.3	19.81	14.1

[a] ETOH: Hydrated ethanol 96% v/v
[b] Conversion factor fresh roots to refined flour 3.5:1
[c] Average production of cassava: 25 t/ha
[d] Real production/Theoretical maximum production

CLAYUCA has also conducted different trials using fresh cassava roots as the feedstock. **Table 3** presents the results of two trials. It can be observed that actual results for the production of hydrated ethanol from fresh cassava did not show great variations among the treatments tested, obtaining a yield of 160 liters per ton of fresh roots. As for the relationship of vinasse/ethanol, the result of 13.6 for the test # 1 indicates a decrease in the number of liters of vinasse that are produced for every liter of ethanol, which is of paramount importance, since the management of this by-product is a critical point in relation to the overall process of bioethanol processing. If more vinasses are generated, it would imply higher costs handling and treating them.

Furthermore, an experiment was conducted at CLAYUCA-CIAT (Del Re *et al*., 2010) to evaluate the effect of the amount of water used on the amount of ethanol and stillage produced. Six fermentation tanks with a capacity of 1,000 liters each were used, in a completely randomized design, replicated in time, with four replicates. The results showed a 37.5% decrease in the amount of water used (500 L vs 800 L), an increase of 107% in ethanol production (44.94 vs 21.75 L) and an increased of 33% in process performance (268.80 L/t compared with 357.50 L/t) (**Table 4**). The increased production efficiency of the process, with the reduction in the amount of water used in the fermentation tanks, was 63% higher than the theoretical value estimated in the evaluation of cassava varieties (375 liters per t versus 220 liters per t), and was very similar to values

used internationally for production of ethanol from cereals (400 liters per t). (Jansson *et al.*, 2009).

Table 3. Results of two tests of hydrated bio-ethanol production from fresh cassava roots at the CIAT-CLAYUCA pilot plant.

	Trial #1	*Trial #2*
Raw material		
Fresh cassava roots (kg)	300	300
Enzymes *(Stargen)* (kg)	0.380	0.380
Yeast (*Ethanol red*) (kg)	0.500	0.500
Urea (kg)	0.300	0.300
Water (kg)	300	450
Products generated		
Hydrated ethanol 96% v/v (L)	48	48
Quantitative analysis		
Total Production (L ETOH)[a]	48	48
Yield (L ETOH/ton roots)	160	160
Yield (L ETOH/ha)[b]	4,000	4,000
Production efficiency ETOH[c]	89%	89%
Ratio vinasses/ethanol (v/v)	13.6	16.7

[a] ETOH: Hydrated etanol 96% v/v
[b] Cassava average production: 25 t/ha
[c] Real production /Theoretical maximum production

Table 4. Ethanol production (L), ethanol efficiency (L per MT of DM), and amount of vinasses produced per liter of ethanol.

	Treatment 1	*Treatment 2*	*Treatment 3*
Raw material			
Fresh cassava roots (kg)	150	150	150
Enzymes *(Stargen)* (kg)	0.714	0.714	0.714
Yeast (*Ethanol red*) (kg)	0.500	0.500	0.500
Urea (kg)	0.350	0.350	0.350
Water (kg)	800	700	500
Products generated			
Hydrated ethanol 96% v/v (L)	21.75 b	27.28 b	44.94 a
Quantitative analysis			
Total Production (L ETOH[a])	21.75 b	27.28 b	44.94 a
Yield (L ETOH/ton cassava flour)	268.80 b	306.60 ab	357.50 a
Ratio vinasses/ethanol (v/v)	25.34 b	19.81 ab	14.09 a

Significant difference for different letters in the same line. Tukey 5%

The analysis made of the quality of the hydrated ethanol produced in the pilot plant of CLAYUCA indicates that the product is a crude, redistilled alcohol for industrial use, which can be easily converted into a neutral rectified spirit, to meet the technical requirements for pharmaceutical use and drinking (**Table 5**).

Table 5. Characteristics of hydrated ethanol produced in the CLAYUCA pilot plant.

Characteristics	Unit	Specification ABNT/NBR (1)	Result
Aspect	-	(2)	Clear
Color	-	(3)	No color
Total acidity (as acetic acid) max.	mg/L	30.0	17.0
% alcohol	% v/v	93.2 ± 0.4	91.3
pH	-	6.0 to 8.0	6.5
Aldehydes (as acetaldehyde) max.	mg/L	60	29
Esters (as ethyl acetate) max.	mg/L	100	47.3
Methanol, máx.	mg/L	500	n.d.
Superior alcohols max.	mg/L	500	163.8

(1) Asociación Brasilera de Normas Técnicas / Brazilian Parameters
(2) Clear and free of wáter or material in suspension
(3) No color to yellow

Energy Balance

Figure 5 shows the energy balance of the process for the production of 250 liters of hydrated ethanol, using cassava flour as the main feedstock. The drying operation of the cassava roots to obtain cassava chips are assumed to be done using solar energy, natural drying. The balance sheet records the electrical energy consumed by each piece of equipment according to the operating time for the production of cassava flour, for the production of bioethanol, and the thermal energy required by the boiler for steam generation. For converting the kw-h to Megajoules (MJ), a conversion factor of 3.6 is used.

The sum of energy consumption indicates that the total electricity consumption was 95.3 kW-h or 342.9 MJ (1 kW-h = 3,600,000 joules = 3.6 MJ), while thermal energy consumption was 3,932.5 MJ. In summary, the total energy consumption (electricity + heat) was 4,275.4 MJ to produce 250 liters of hydrated ethanol; therefore, energy consumption for producing one liter of ethanol in the bio-refinery is 17.10 MJ.

If a value of 1.54 MJ/L is used to indicate the major agricultural operations required to produce a liter of ethanol from cassava (Assis, 2008), a total value (agronomic + industrial consumption) of 18.64 MJ/L is reached. Considering that one liter of ethanol is equivalent to an energy value of 23.375 MJ, then a positive energy return rate of 1.25 is obtained. For every unit of energy invested, 25% more energy is obtained.

Production costs for hydrated ethanol

Based on data obtained in the biorefinery model developed by CLAYUCA-CIAT (500 liters per day), the total cost of production of hydrated ethanol (96% v/v) was estimated at $ 1.34 per liter, which includes costs of raw material, processing, depreciation and maintenance, in addition to any gains from the sale of co-products (**Table 6**).

Table 6. Estimated production costs of hydrated bioethanol from cassava, CLAYUCA pilot plant.

Item	Cost (USD[a])	
	Per liter	*%*
Raw material		
Cassava roots (0.055 USD/kg)	0.51	38.0
Production of cassava flour		
Electricity	0.02	1.5
Labor	0.06	4.5
Production of ethanol		
Water	0.01	0.7
Electricity	0.02	1.5
Wood	0.04	3.0
Reagents	0.41	30.6
Labor	0.06	4.5
Subtotal Process	**1.13**	
Sales of by-products[b]	- 0.08	
Depreciation and maintenance[c]	0.29	15.7
Total Cost of Production	**1.34**	100.0

[a] 1 USD = 1.800 Colombian pesos
[b] Cost recovery for sales of by-products (375 kg at 0.11 USD/kg)
[c] Depreciation 5 years, 250 days/year. Maintenance: 4% per year. Initial investment: 150,000 USD

Management of effluents (vinasses)

Any processing operation to obtain ethanol will have as one of the most sensitive aspects of environmental impact and energy consumption and cost, the large amount of effluents that are produced as waste from the process. On average, for every liter of ethanol obtained, between 10 and 15 liters of effluents, also known as vinasses, will be generated. The effluents are the organic liquid by-product resulting from the fermentation of carbohydrates (sugarcane juice, molasses, cassava slurry) and after distillation of fermented mash. The composition of the effluents is variable and depends on the characteristics of the raw material used in the production of alcohol, and the type and efficiency of the fermentation and distillation steps. In general, the effluents are composed of water,

minerals, organic matter, residual yeast and non-fermentable constituents. **Table 7** presents the chemical composition, *in vitro* digestibility of dry matter, organic matter content and starch of cassava effluents obtained from the fermentation of fresh cassava. **Table 8** presents the concentration of minerals, on a dry basis.

Table 7. Chemical composition of vinasses from cassava-based ethanol production.

[1]Crude protein	[1]Ashes	[1]Ethereal extract	[1]Crude fiber	[1]Moisture	[2]IVDDM	[2]OM	[3]Starch
11.60%	5.23%	4.86%	60.35%	8.49%	64.70%	93.52%	0.74%

[1] Analytical Services Laboratory LSA. CIAT, 2008
[1] Forages Laboratory. CIAT, 2008.
[2] IVDDM: in vitro digestibility of dry matter
[2] OM = organic matter
[3] Cassava Quality Laboratory. CIAT, 2008

Table 8. Mineral content present in the effluents from cassava-based ethanol production.

P	**K**	**Ca**	**Mg**	**S**	**Zn**	**B**	**Mn**	**Fe**	**Cu**	**Al**	**Na**
%	%	%	%	%	ppm	ppm	ppm	ppm	ppm	ppm	ppm
1.42	1.49	5.38	0.40	0.48	40.4	15.5	104.5	3,305.1	14.2	3,120.6	38,398.2

Source: *Analytical Services Laboratory, CIAT. CLAYUCA, 2008*

The concentrations of minerals in the effluents of cassava ethanol are low with the exception of Ca (5.38%), limiting its use as a single product. Most of the chemical components found in the stillage are in the form of chelates, which allows the formation of complex organic nitrogen and other minerals that have better bio-availability in animal nutrition. The typical chemical composition of cassava ethanol effluents includes components such us: soluble inorganic substances (K, Ca and SO_4), dead cells, yeast, organic substances resulting from the metabolic processes of yeast and microorganisms, alcohol and residual sugar, insoluble organic substances and volatile organic substances, which may be useful in developing various products, especially for animal feeding programs (Ospina *et al.,* 2008).

Vinasses are among the largest organic waste polluting effect on the flora and fauna of the planet, since they have a high content of organic matter, measured as chemical oxygen demand (COD) and Biological Oxygen Demand (BOD), which are in a range of 24,635 to 65,457 and 26,500 to 33,600 mg O_2/L, high concentrations of fixed soluble solids (1,400-2,000 mg/L), very low pH (3.6-3.8), high concentrations of phenol (478 to 541 mg gallic acid/L), and phosphate and sulfate contents in the range of 290 to 1,705 mg/L and 308 to 946 mg/L, respectively (Robles and Villalobos. n.d)

For the treatment and utilization of effluents generated in the production of ethanol, there are no simple techniques of bioremediation (filtering) that allow complete environmental standards, as most of the solids are in solution with very small particle sizes. In the RUSBI methodology, the treatment of effluents is done by using biopolymers, which are electrically charged chemical compounds, produced from starch. When the biopolymers come into contact with solutions of high ionic solid loads and a basic pH, a flocculation and coagulation process is enhanced. After the organic matter contained in effluents is flocculated and coagulated, the resulting wet sludge is removed, and the clarified water can be used in other activities of the distillery or for irrigation purposes.

To flocculate and coagulate the vinasses, the polymers are prepared at a concentration of 1,000 ppm. The products obtained are called clarified vinasses and clarified sludge. **Figure 6** and **7** illustrate the process for decanting the solids from the effluents and **Table 9** shows the nutrient content present in each of the clarified product.

Table 9. Composition of sugar cane vinasses, clarified vinasses and clarified sludge.

	P total	K total	Ca total	Mg total	S	Fe	Cu	Na	Zn	Prot	OM
			(%)				(mg/kg)			(%)	(%)
Sugarcane vinasses	2.97	10.24	0.88	1.14	1.23	986	6.0	3,066	54.0	6.95	56.83
Clarified vinasses	0.00	1.06	0.48	0.12	0.14	32	0.0	366	3.0	0.81	6.79
Clarified sludge	2.75	2.99	14.26	0.20	9.30	525	47.0	467	19.0	5.15	27.51

Source: *Analytical Services Laboratory, CIAT. CLAYUCA, 2007*

CLAYUCA-CIAT in partnership with a private company in USA (Soil Net[6]) and one Brazilian University (UFRGS[7]) have developed new potential solutions and alternatives for sustainable, competitive management of the effluents generated in the biofuel distilleries. One of these alternatives is the development of protein and energy supplements for ruminants, mixing the vinasses with cassava products (roots and foliage). The nutritional supplements developed with vinasses, have been oriented principally to feed ruminants. The products can have different compositions and characteristics, depending on the type of animal to be fed.

The organic matter contained in the flocculated sludge is mixed with other products and co-products obtained during the process such us cassava and sweet potato leaves and stems, and sweet sorghum bagasse. Other components that are included are urea, minerals and additives. The formulation of the nutritional supplement is scientifically designed with the help of a computer program to obtain a final product that is competitive, nutritionally balanced and highly efficient in the feeding of ruminants. **Figure 8** presents the different

[6] Soil Net LLC, Polymers Solutions. Wisconsin, USA. www.soilnetllc.com
[7] Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil

steps required to prepare the nutritional supplement. In the preparation of the multinutritional blocks, the ingredients (bagasse, molasses, vinasse, urea, sodium bentonite and minerals) are previously weighed and mixed in a horizontal mixer. The order of introduction of ingredients is defined to avoid losses of molasses in the walls of the mixer and enhance chemical reactions and heat generation, to ensure the compaction of the blocks. First, the bagasse, minerals and sodium bentonite are mixed. Then, a solution of urea, diluted in the vinasse and molasses is incorporated in the mix. Finally, the calcium oxide is incorporated. The mixture is agitated for 15 minutes until a homogeneous appearance is obtained. The formation of the blocks is done taking 18 kg of the mixture, and placing this in an iron bowl to receive a compaction pressure of 2000 kg/cm^2, for 5 minutes. Finally, the blocks are removed from the containers and placed in a shaded area for drying, during one week. For transportation and commercialization, the blocks are packed in cardboard boxes.

In the preparation of supplements especially for cattle feeding, co-products from sugarcane-based ethanol can be included between 50 and 80 percent. **Tables 10** and **11** present the components and nutritional composition of two products: a multinutritional block and a mineral salt, for ruminants, made with co-products from sugarcane-based biofuel processing, following the process indicated above. **Table 12** presents the bromatological composition of the two nutritional supplements (energy and protein), elaborated in the form of blocks and salts.

Table 10. General characteristics of a nutritional supplement block.

Raw material	Level of inclusion (%)	Nutritional composition (%)	
Pre-digested bagasse	25.10	Crude protein	24.00
Vinasse sludge	36.82	NPN (max.)	3.85
Fly ash	4.32	TDN	33.00
Molasses B	9.89	Ca	2.21
Other Ingredients	23.87	P	1.00
Total	100.00	S	0.36
Other ingredients: Urea, NaCl, sulfur flower, dicalcium phosphate, calcium oxide, sodium bentonite, micronutrients nucleus		NPN = Non Proteic Nitrogen TDN = Total Digestible Nutrients	

***Source**: CLAYUCA, 2009.*

Table 11. General characteristics of the nutritional supplement salt.

Raw material	Level of inclusion (%)	Nutritional composition (%)	
Pre-digested bagasse	24.45	Crude protein	24.00
Clarified sludge	35.86	NPN (max.)	0.90
Filter cake	4.63	TDN	34.00
Molasses B	9.90	Ca	2.21
Other Ingredients	25.16	P	1.00
Total	100.00	S	0.36

Other ingredients:
NaCl, sulfur flower, dicalcium phosphate, sodium bentonite, urea, mineral nucleus.

NPN = Non Protein Nitrogen
TDN = Total Digestible Nutrients

***Source**: CLAYUCA, 2009.*

Table 12. Bromatological composition of two nutritional supplements (energy and protein), elaborated as blocks and salts, using vinasse and other products and co-products from sugarcane-based biofuel processing.

Nutrients	Protein		Energy	
	Block	*Salt*	*Block*	*Salt*
Dry matter (%)	78.01	93.44	78.99	94.15
Organic matter (%)	67.59	59.43	67.67	65.04
Crude protein (CP) (%)	33.07	39.51	9.61	17.20
Fat	0.82	2.20	1.30	1.59
TDN	65.54	64.26	69.91	65.54

***Source**: Ruminants Nutrition Laboratory-LANUR. UFRGS, 2007*

Another feature of the elaboration process of the nutritional blocks is the increase in crude protein content, with increased levels of vinasse, in the formulation of the product. This change occurs due to the presence of yeast residues in the vinasse that enrich the nutritional value of the product (Loaiza, 2008). These positive features make the nutritional blocks a very attractive product, with great market potential in the animal feed sector. **Figure 8** shows the acceptance of the product by the animals.

Bio-economic animal feeding trials with the nutritional supplements

The quality and efficiency of the nutritional supplements made with co-products and effluents from sugarcane-based and cassava-based ethanol processing, has been tested in bio-economic animal feeding trials.

In a commercial test with calves with initial weight less than 200 kilograms, a nutritional supplement was fed during 90 days, to complement a grass-fed basal diet with *Penissetum purpureum.* Average weight gains per animal per day of 0.602 kilograms were obtained, higher than the average weight gains obtained by the animals before starting the supplementation, 0.316 kg/animal/day. The short duration of this feeding trial does not allow main conclusions to be made. The main objective of this experiment was to make a first evaluation about the acceptance of the block by the animals and to have an initial estimate of the consumption potential. The animals in the study consumed the block from the first day of exposure, without any rejection related to the smell or taste of the product. The nutritional block retained its structure during the whole supplementation period.

Another trial aimed at assessing the consumption and weight gain of heifers on pasture, supplemented with protein supplements prepared from cassava roots and leaf flour, and vinasse from sugarcane-based biofuel production (Gil *et al.*, 2007). The study included 20 heifers of replacement of the Holstein breed with an average initial weight of 168 kg, divided into two groups of 10 animals each. One group was used for evaluating the protein supplement based on cassava and vinasse, and the other to assess the use of a commercial supplement. The experiment lasted for 120 days (September-December). Four grazing plots planted with African star grass (*Cynodon nlemfluensis*), with an average area of 5,518.5 m^2 for each plot were used for the rotation of the animals (each group used two pastures). The forage dry matter on offer was on average 2,320.5 kg for each grazing plot, equivalent to 4,204.9 kg of dry matter per ha. The trial was conducted in a region near Palmira, Valle, Colombia.

Animals were distributed randomly into two groups: the first group received 1.5 kg/day/animal of a commercial concentrate (18% of protein and 67% of TDN) and the second group received 1.0 kg/day/animal of supplement based on cassava and vinasse (21% protein, and 56% of TDN). The group receiving the vinasse-based supplement was given a period of 10 days to get used to the product. Weighing was conducted every 21 days and supplement consumption assessed, taking into account the daily supply of supplement. The commercial supplement and the supplement based on cassava were weighed in the morning. In the afternoon, the feeders were reviewed to collect and weigh the wastes or leftovers. In both cases, the consumption of supplements was complete. The assessment of the weight gains indicated that those animals that consumed the supplement of cassava and vinasse presented a better performance than the animals fed with commercial product. Weight gains were on average 0.48 kilograms per day whereas with the commercial concentrate, the weight gains were on average 0.36 kilograms per day ($p<0.05$).The slightly higher weight gain obtained by the animals consuming the cassava-based supplement could be explained by considering the higher protein content of the

cassava-based supplement and the better ratio of nutrients (rumen degradable protein/TDN).

Another test was developed in the Cauca River Valley of Colombia. The experimental area consisted of 17 paddocks divided with electric fences, each with an approximate area of 0.25 ha, planted with African star grass (*Cynodon plectostachyus*). Each paddock had an automatic water supplier and a feeder for the nutritional supplement. Rotational grazing was used with about 2 days of occupation and 17 days of rest. The pastures were fertilized with 80 kg P_2O_5 per ha per year, and 50 kg N per ha per year. During the dry season, the pastures were uncompacted and irrigated. A total of 71 steers of undefined breed, aged approximately 24 months, and with an initial average live weight of 234 kg were used (**Figure 11**). The treatments evaluated consisted of a conventional mineral supplement and a protein-mineral block supplement (**Table 11**).

Table 11. Composition of nutritional supplements offered to cattle.

Nutrient	Conventional mineral salt (6%)[1)]	Nutritional blocks
Crude protein (%)		24
Non Protein Nitrogen (NPN)(% máx.)		3.85
Total Digestible Nutrients (TDN) (%)		33
Sodium Chlorine (g/kg)	3852	19.62
Phosphorus (g/kg)	6.0	10.04
Calcium (g/kg)	12.0	22.12
Magnesium (g/kg)	0.5	1.91
Sulfur (g/kg)	6.0	3.60
Copper (ppm)	2500	82
Zinc (ppm)	8000	247
Iodine (ppm)	150	5.96
Cobalt (ppm)	40	0.82
Selenium	100	0.82

[1)] http://www.somexnutricion.com
Source: *CLAYUCA, 2009.*

Statistical analysis of the data obtained indicated that daily weight gains of animals consuming the nutritional blocks was 21% higher than the weight gains obtained by the animals consuming the mineral supplement ($P<0.05$) (**Table 12**). The weight gains obtained indicate the potential of the nutritional supplements for use in animal feeding. The analysis of economic efficiency was also positive. The average daily weight gain of animals consuming multinutritional blocks was 94 grams per day (6%) higher than that of the animals that were supplemented with mineral salt. This improved efficiency represented a 17% increment in the gross margin ($1251.2 vs. $ 1072.4), making it an attractive option for cattle producers (**Table 13**). The objective of this experiment was to validate the option of elaborating a nutritional supplement that could give the animal not only the minerals, but also the protein and some energy. The questions that this experiment was trying to answer was whether it would be possible to have a complete nutritional supplement (minerals,

energy, protein) that was competitive in relation to the mineral supplements that were available in the market.

Table 12. Initial and final live weight, and the average daily weight gain of grazing animals given nutritional supplementation.

Variables	Treatments	
	Multinutritional block	Mineral Salt 6%
Initial weight (kg)	231.47	235.39
Final weight (kg)	273.15	269.83
Weight gain (g/day)	541a	447b

a,b Tukey test (P<0.05). ***Source:*** *CLAYUCA, 2009.*

Table 13. Weight gains and economic benefits from grazing animals given nutritional supplementation.

Parameters	Multinutritional block	Mineral salt 6%
Average initial weight (kg)	231.0	235.0
Average final weight (kg)	273.2	269.8
Average weight gain (kg)	42.20	34.80
# days of trial	78	78
Average daily weight gain (kg/day)	0.541	0.446
Price live kg (Col $)	2655	2655
Price average daily weight gain (Col $)	1436.4	1184.1
Supplement consumed (kg/day)	0.177	0.071
Nutritional supplement consumed (Col/kg)	1046.6	1565.02
Costs of nutritional supplement (Col $)	185.2	111.7
Gross margin (Col $)	1251.2	1072.4
Gross margin (US $)	0.622	0.533

Source: *CLAYUCA, 2009.*

Economic viability of the use of nutritional supplements in animal feeding

The economic viability of the use of nutritional supplements for animal feeding, based on the by-products and co-products from sugarcane and cassava biofuel operations, will depend on the cost of producing the nutritional supplements and the price competitiveness in relation with the price of similar products available in the commercial market.

In the Colombian sector of cattle producers, the use of nutritional supplements is practiced, although the percentage of cattle growers that uses them is still limited. In some cases, the transportation costs to the areas with large cattle operations increases the final costs of the nutritional supplements. The products commercially available are presented in the form of blocks, with a weight of 25 kilograms each, usually including molasses and urea. As of August, 2011, the cost of a multi nutritional block was $ 28,000, Colombian pesos. This value is equivalent to US$ 15.55, considering the average exchange rate (1 US$ equal to Col $ 1,800 in August 2011). The cost per kilogram of nutritional block is US$ 0.622. The nutritional block that is available in the market is 52% more expensive than the product produced with the RUSBI approach. This large margin indicates a tremendous market potential for these nutritional supplements in the animal feed sector.

The technical and economic feasibility of using by-products and co-products from a sugarcane- or cassava-based biofuel operation, to elaborate nutritional supplements for animal feeding, has been demonstrated. It is possible to use the nutritional supplements in animal feeding programs with good results of biological and economic efficiency parameters. It is also feasible to establish market linkages with the animal production sector and to position the nutritional supplements, based on the competitive price of producing them as compared with the products that are commercially available. However, the work conducted by CLAYUCA and collaborating agencies, institutions and private sector companies, has been focused in the context of a strategy designed to promote biofuel production and use by small-scale communities and farmer groups, the RUSBI approach. In this sense, the initial beneficiaries of the technology developed for the preparation and use of the nutritional supplements will be the commercial groups that are already operating the bio-ethanol distilleries, with large volumes of effluents that need to be managed with economic and environmental efficiency. The small-scale rural communities, cooperatives and farmer groups that the RUSBI approach is targeting, will not be able to compete with the large scale biofuels distilleries and sugarcane operations. The objective of the RUSBI approach is not to enter into this market. What RUSBI aims to achieve is to aggregate value to the biofuels that can be produced by small-scale farmers, promoting its use locally, for their own consumption, or for commercializing it in local markets, promoted by the government (social ethanol), or by private sector initiatives. The sustainable, competitive management of the effluents becomes an additional component of this approach, with potential to help farmers improve the feeding systems of their animals, and increasing their incomes.

CONCLUSIONS

Lack of access to energy is a great barrier for economic development and growth, especially in isolated areas in which the installation of the electric grids is very expensive. The approach developed by CIAT and CLAYUCA, known as Rural Social Biorefineries-RUSBI, for the production and local use of biofuels, based on feed stocks that can be produced easily by farmer groups and rural communities, is a viable option for countries and regions with limited access to modern forms of energy. In these situations, small-scale

biofuel production and use can help to improve access to energy with positive effects on rural development, and poverty and hunger alleviation.

Results obtained by CIAT and CLAYUCA, in the evaluation of cassava as a feedstock for the production of hydrated ethanol, suggest that there is a huge potential to explore the genetic diversity of cassava and optimize the transformation process from biomass to ethanol. Additional studies are required on mass and energy balances and bio-economic efficiency to confirm and strengthen the economic viability of cassava as a feed stock for ethanol production.

One of the most important components of the RUSBI approach is the innovative management of the effluents and different products and co-products generated during the biofuels production process, converting them into nutritional supplements for animal feeding, especially for cattle. This activity helps to improve the overall economic efficiency of the process and has positive impacts on the environment. The RUSBI approach uses the flocculation with biopolymers for treating the effluents and the acceptance and assimilation of this technology by the farmer groups has to take into account the specific context of the target groups, usually with limited financial resources to invest, and with low educational levels to handle and assimilate sophisticated processes and technologies. The technologies offered have to be simple, efficient and sustainable.

The incorporation of the RUSBI concept in the production of biofuels has the potential to revitalize the social inclusion programs, adding value to their agricultural production systems and promoting socio-economic development of their communities.

Social rural biorefineries can become the key components for the future development of integrated models of food production, raw materials and fuel, especially at the level of small-scale rural communities, located in marginal areas, with little access to conventional sources of energy. Improved access to energy sources could be the first step towards rural development processes through which farmer groups can gain more control over their natural resources and can have more participation in the distribution of benefits generated in the biofuel value chain.

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ABBREVIATIONS AND ACRONYMS

ASL:	Analytical Services Laboratory
BOD:	Biological Oxygen Demand
CIAT:	International Center for Tropical Agriculture
CLAYUCA:	Latin American and Caribbean Consortium to Support Research and Development of Cassava
COD:	Chemical Oxygen Demand
LANUR:	Laboratório de Nutrição de Ruminantes
LSA	Analitical Services Laboratory
TDN:	Total Digestible Nutrients
NNP:	Non Proteic Nitrogen
RUSBI:	Rural Social Bio-refineries
SHF:	Simultaneous Hydrolysis and Fermentation
SOIL NET LLC:	Polymers Solutions. Private Company in USA
UFRGS:	Universidade Federal do Rio Grande do Sul
UNDESA:	United Nations Department of Economic and Social Affairs
UNDP:	United Nations Development Programme

Photo 1. Illustration of an unusual plant type observed in an S1 family. Leaves lack petioles, and there is restricted branching in most of the plants expressing this recessive trait.

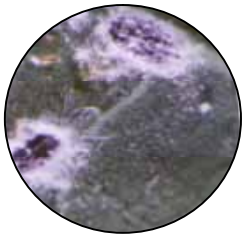
A. FOLIAGE FEEDERS

1. Whiteflies

Adults of *Aleurotrachelus socialis*

Eggs

Nymphs



Pupae

Bemisia tabaci: Adults, eggs and pupa

Symptoms of African cassava mosaic virus transmitted by *Bemisia tabaci*

Adults of *Aleurodichus dispersus*

Eggs laid in spiral form

Damage: Leaf curling, chlorosis and leaf size reduction

Host Plant Resistance. This form of resistance to whiteflies is rare in cultivated crops.

White fly cassava resistant variety Nataima 31.

Root production

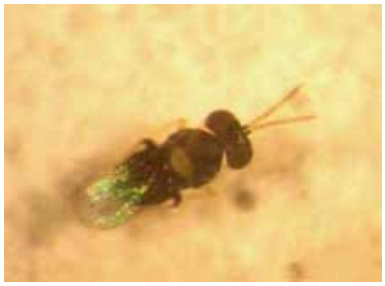
Biological control.

Encarsia hispida

Eretmocerus sp.

Amitus macgowni

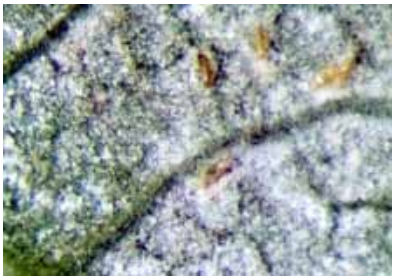
Aleuroctonus vittatus



Encarsia sp.

Euderomphale sp.

More than 20 species of entomopathogens have been reported infecting whiteflies on cassava,



Eggs

Nymphs

Pupae

Different stages of *A. socialis* affected by *Lecanicillium* (*Verticillium*) *lecani*

The most frequently observed predators feeding on cassava whiteflies are crysopids (Neuroptera: Chrysopidae).

Eggs of *Chrysopa* spp

Nymph of *Chrysopa* spp

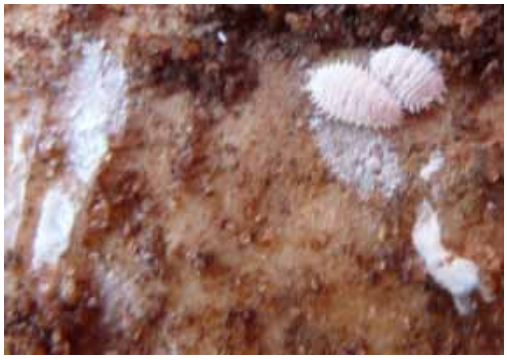
Adult of *Chrisopa* spp

2. Cassava Mealybugs

Phenacoccus herreni female

P. herreni pupae and male adult

P. herreni female adult with ovisac



Dysmicoccus sp.: A specie of mealybug attacking cassava roots in Brazil

Damage

Mealybug damage

Biological control

Anagyrus lopezi parasitoid of *P. manihoti*

Acerophagus coccois

Anagyrus diversicornis

Aenasius vexans

Parasitoids of mealybugs found in Colombia and Venezuela

3. Cassava Mites

M. tanajoa ♀

M. tanajoa ♂

M. caribbeanae

Tetranychus urticae

Tetranychus cinavarinus

Damage

Damage by *Mononychellus tanajoa*

Damage of *Tetranychus urticae*

Host plant resistance

Host plant resistance to *Mononychellus tanajoa*

Biological control

Phytoseiids predators of mites

Staphylinid: *Oligota minuta*

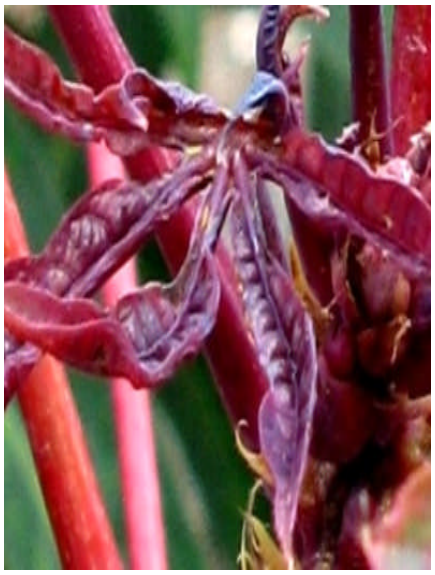
Coccinellid: *Stethorus* sp.

Fungal pathogen of mites: *Neozygites* sp. (Zygomycetes: Entomophthorales)

4. Thrips

Scirtothrips manihoti

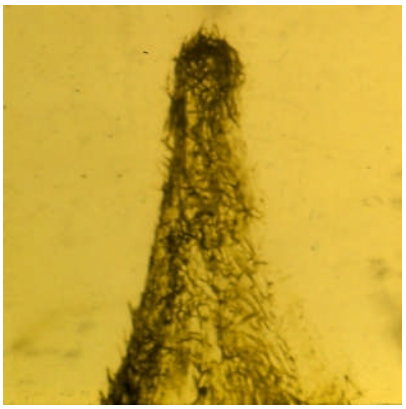
Corynothrips stenopterus



Thrips on growing points and young leaves

Severe damage by thrips

Susceptible: non-pubescent cultivar



Resistant: pubescent cultivar

5. Cassava Lacebugs

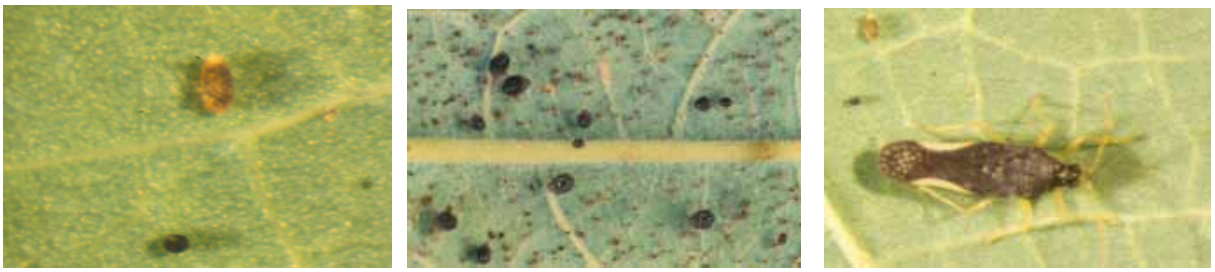
Eggs

Nymph

Adult

Biological stages of the lacebug (*Vatiga* spp)

Amblystira machalana,



Eggs Nymphs Adult

Biological stages of the black lacebug (*Amblystira machalana*)

Damage

Damage of lacebugs

6. Cassava Hornworms


E. ello. Adult in normal position and larva

Damage

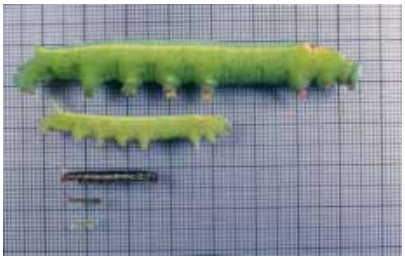

E. ello damage


Female adult


Male adult


Green egg


Yellow egg


Five larvae instars



Prepupa and pupa in the soil

Management

Black light traps

Adults captured

Field evaluation of eggs

Trichogramma spp.

Telenomus sphinguis

Cotesia spp.

Diptera; Tachinidae

Parasitoids of eggs and larvae

Chrysopa spp. predator of eggs and larvae

Polistes erythrocephalus

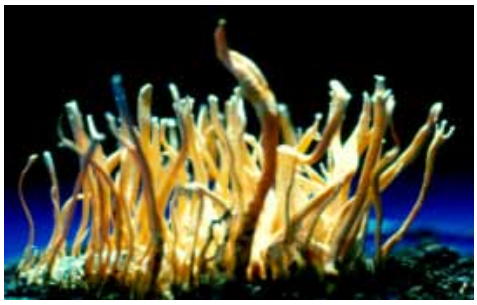
Spider

Podisus nigrispinus

Eggs and larvae predators

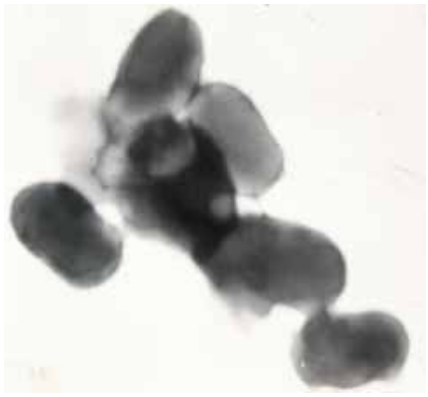
Important entomopathogens include *Cordyceps* sp. (Aconycites: Clavicipitaceae),

Cordyceps sp. affecting *E. ello* pupae



Cordiceps sp. emerging from the soil

A granulosis virus of the family Baculoviridae was found attacking *E. ello* on cassava



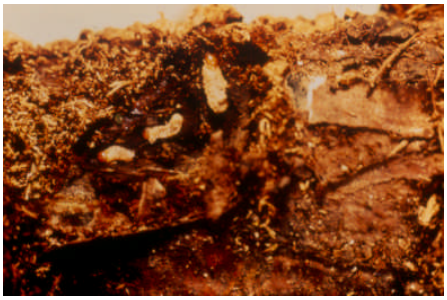
Larvae affected by *Baculovirus*, extraction and viral bodies

B. STEMBORERS AND STEM FEEDERS

7. Stem Borers

Larvae of *Coelosternus* spp

Adult



Damage

Damage of *Chilomima clarkei*





C. clarkei: Adult Eggs Larva Pupa Damage



Brachymeria conica *Tetrastichus howardi* Entomopathogen: *Spicaria* sp.

Parasitoids and entomopathogens of *Chilomima clarkei*

8. Scale Insects



White scale: *Aonidomitylus albus* Black scale: *Saissetia miranda*

9. Fruitflies

Adult of the cassava fruit fly

External and internal damage of the stem

Stem rot

Fruit damage

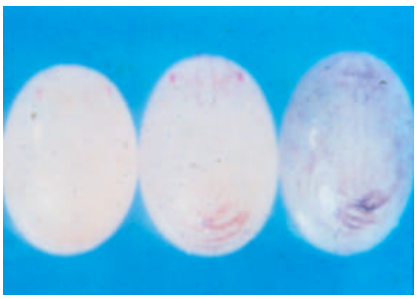
Damage by the fruitfly in the cassava stem and fruit

10. Shootflies

Shootflies *Neosilba* sp. Damage, adult and larvae

C. SOIL-BORNE PESTS

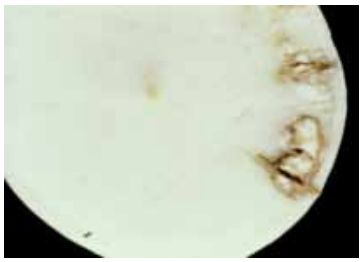
11. Cassava Burrower Bug



Cyrtomenus bergi: Eggs Nymphs Adults

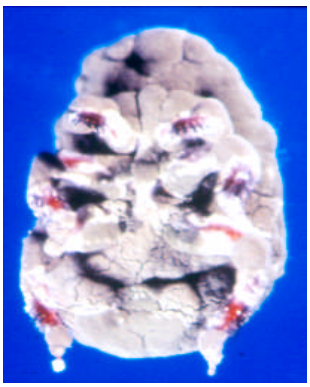

Cassava burrower bug. On maize and onions

Damage.



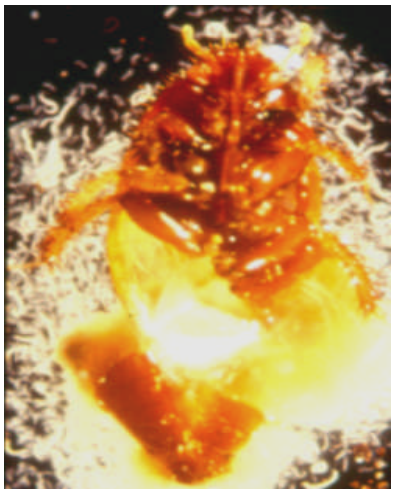
Damage by *Cyrtomenus bergi*: Local rotted spots on the root parenchyma.

Biological control

Biological control of *C. bergi* with *Metarhizium anisopliae*

Several species of nematodes



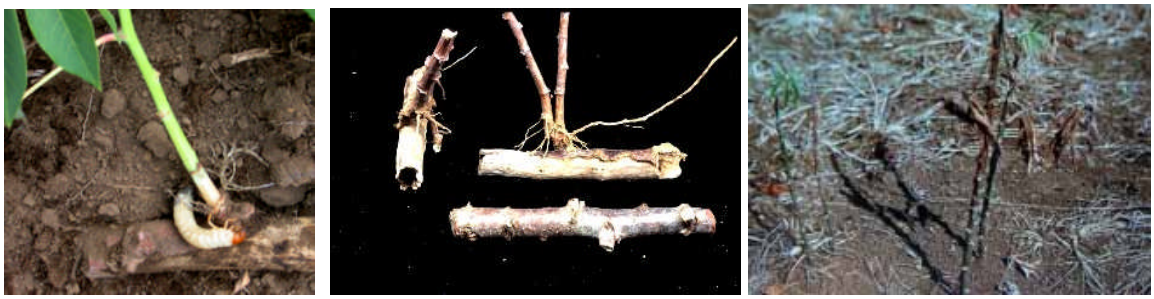
Nematodes parasitizing *C. bergi.*

12. White grubs


Larva of white grubs

Adults of white grubs

Damage.



White grubs feeding on stem cuttings of cassava

Numerous microbial agents for the biological control of white grubs have been identified

Bacillus popilliae

Metarhyzium sp.

D. SECONDARY PESTS

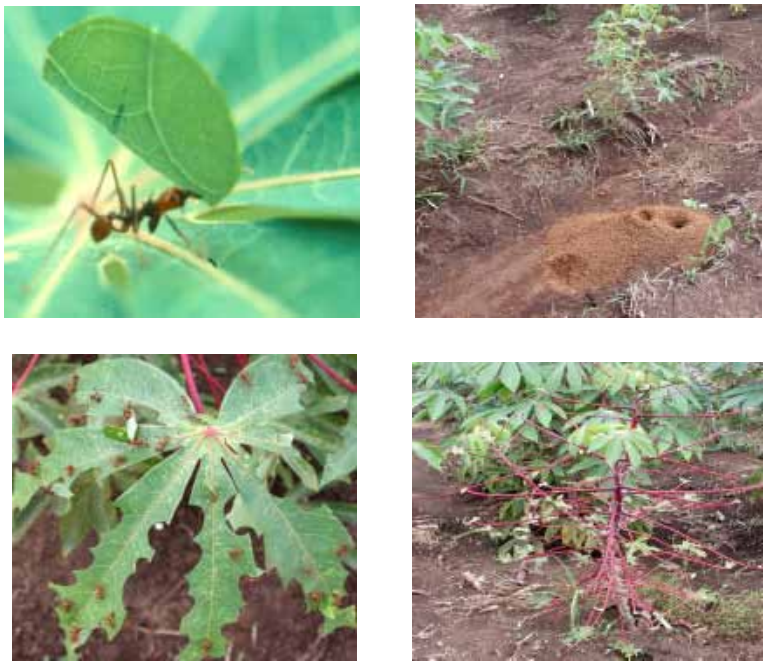
14. Grasshoppers (*Zonocerus elegans* and *Zonocerus variegatus*)

Grasshoppers feeding on cassava

15. Gall Midges *Iatrophobia brasiliensis*

Gall midges: *Iatrophobia brasiliensis*

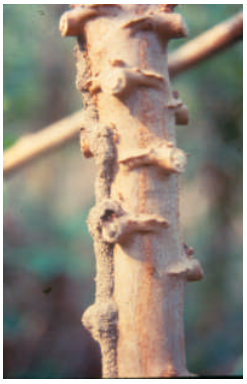
16. Leaf-cutter Ants



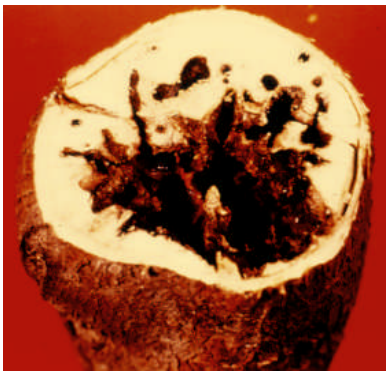
Damage of leaf-cutter ants

17. Termites

Termites: Soldier



Termites on the stake



Root damage by termites

18. Leafhoppers

Perkinsiella saccharicida

Scaphytopius margelineatus

Empoasca bispinata

Peregrinus maidiz

Leafhoppers on cassava

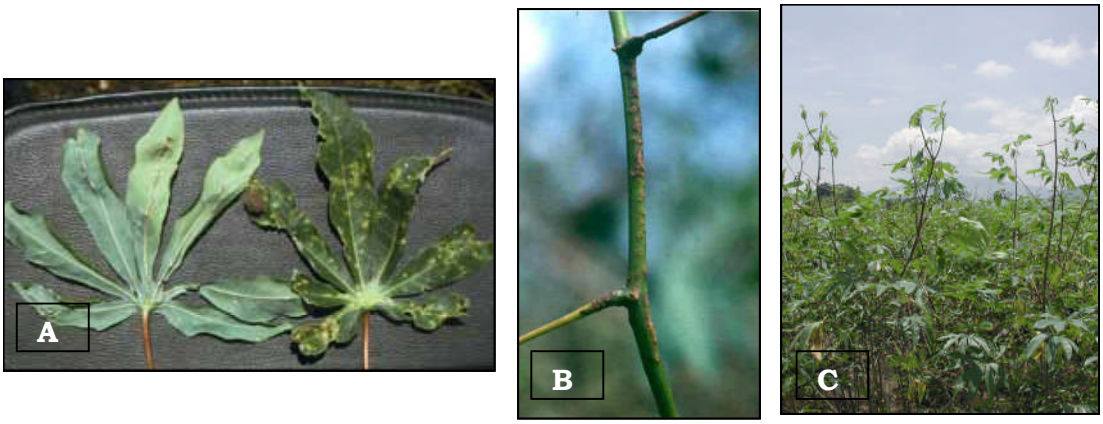


Figure 1. Symptoms of superelongation disease in cassava: (A) cankers on leaves, B) cankers on petioles and stem, and (C) elongated stem.

Figure 2. Leaf spots caused by Cercospora henningsii.

Figure 3. Leaf spots caused by Cercospora vicosae in a cassava leaf.

Figure 4. Leaf spots caused by Phaeoramularia manihotis.

Figure 5. Leaf spots caused by Phoma sp. in cassava.

Figure 6. Cassava ash symptoms, caused by Oidium sp.

Figure 7. Leaves and stem show cankers caused by Glomerella manihotis.

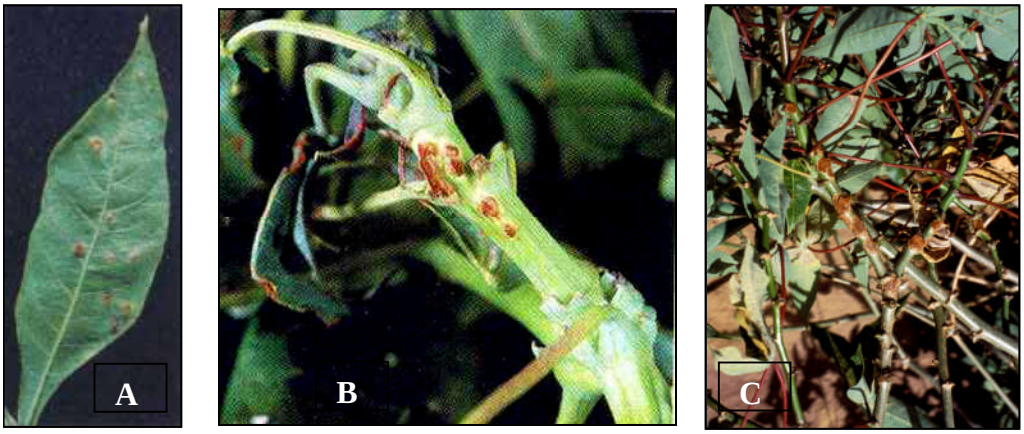


Figure 8. Symptoms of cassava rust characterized by pustule formation on (A) leaf, and (B) and (C) stems.

Figure 9. Necrosis caused by Glomerella cingulata in cassava stems.

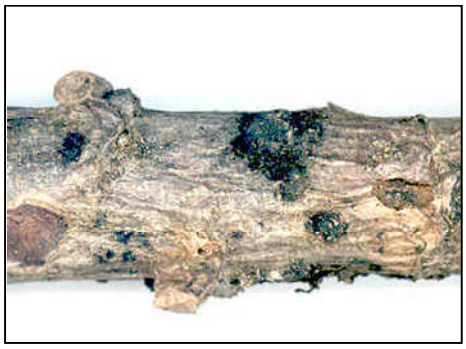


Figure 10. Stem rot in a stake infected by Diplodia sp.

Figure 11. Rot caused by Rosellinia necatrix in cassava roots.

Figure 12. Cassava root rot symptoms have been observed in Rayong and at the Thai Tapioca Development Institute (TTDI) in Huay Bong, Nakhon Ratchasima, Thailand.

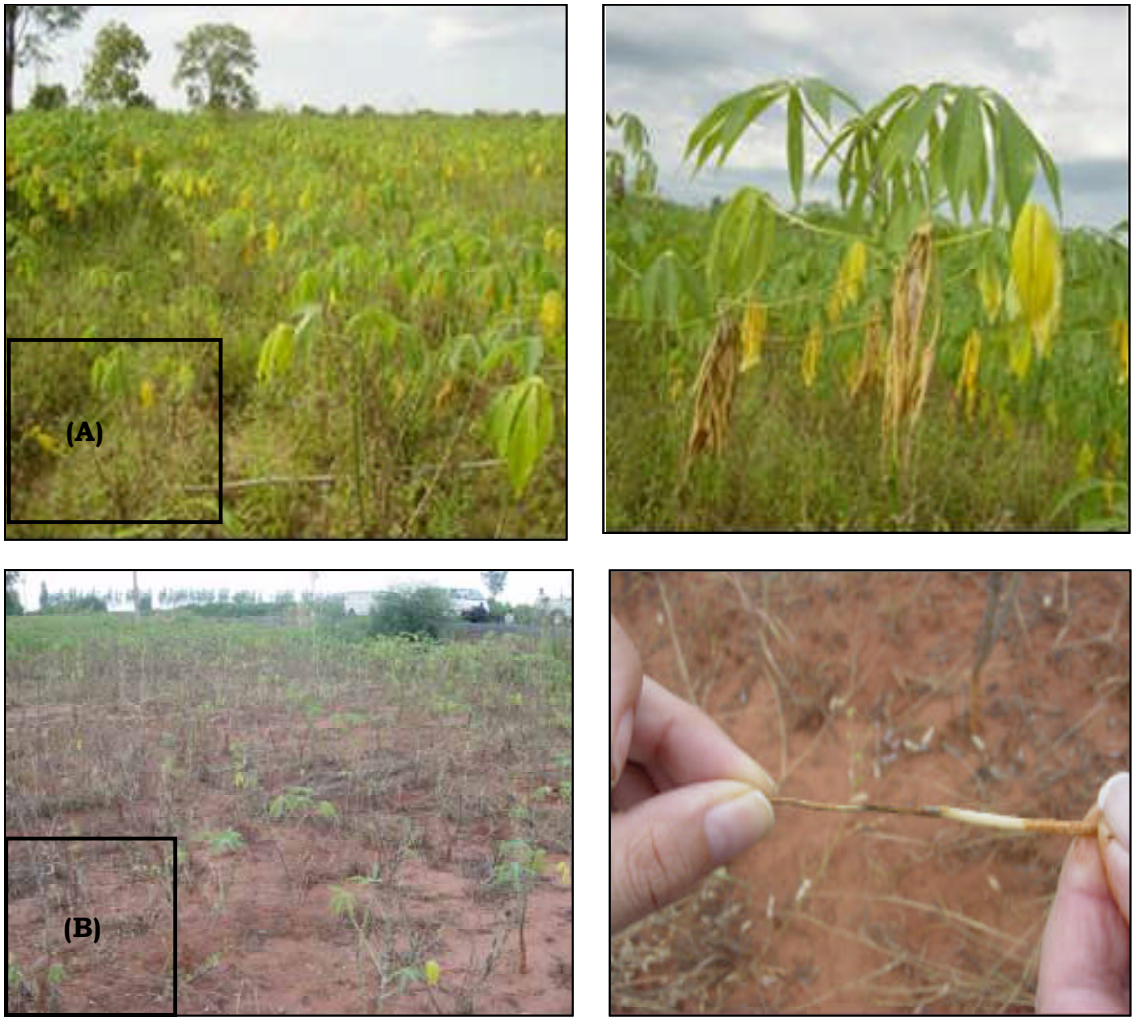


Figure 13. Cassava plants showing symptoms of root rots and wilting in Buriram province (A) and Nakhon Ratchasima province (B) of Thailand.

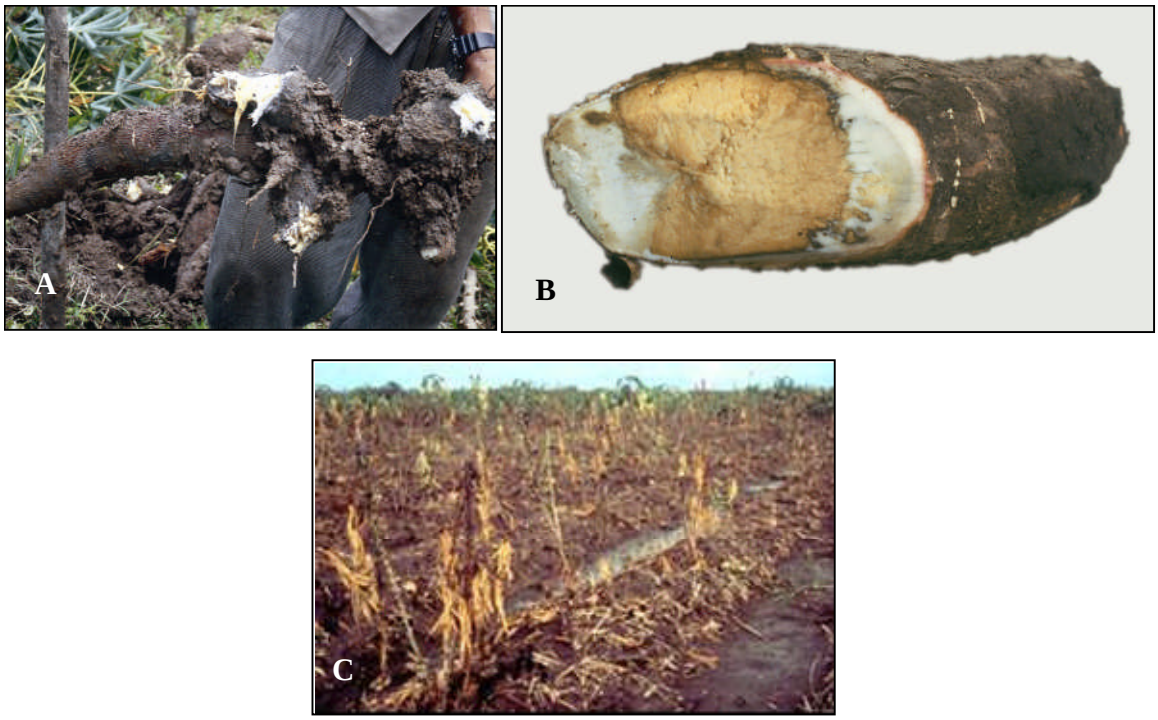


Figure 14. Root rots (A and B) and plant wilt (C) caused by Phytophthora spp.

Figure 15. Cassava Bacterial Blight (CBB) symptoms observed on cassava leaves of cv. Rayong 5 in Thailand.

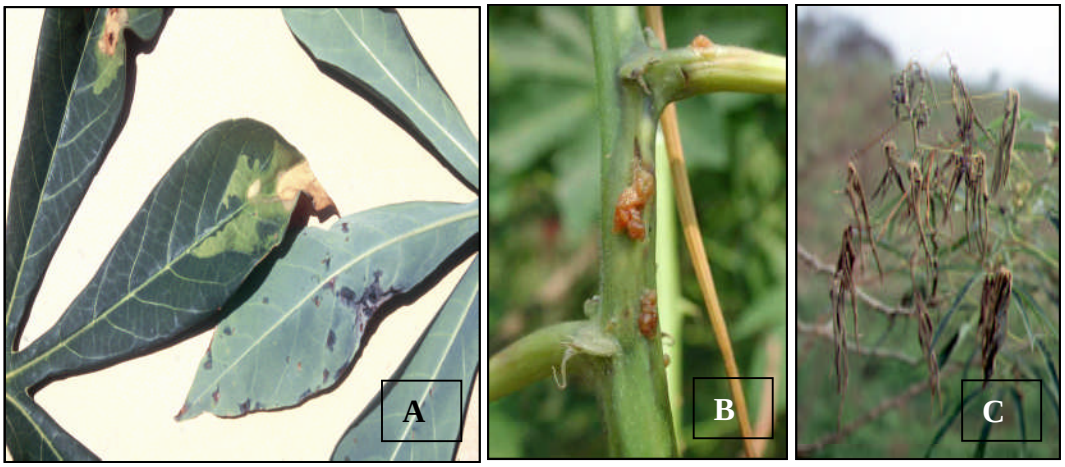


Figure 16. Symptoms of cassava bacterial blight, induced by the bacterium Xanthomonas axonopodis pv. manihotis: (A) angular leaf spots and leaf blight, (B) exudate on stem, and (C) plant wilt.

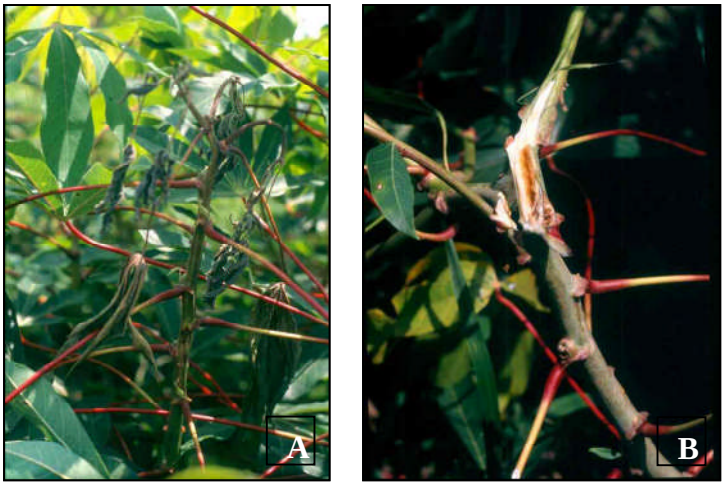


Figure 17. Symptoms caused by Erwinia carotovora: (A) wilt, and (B) damage to the medulla.

Figure 18. Galls on stem caused by Agrobacterium tumefasciens

Figure 19. Symptoms of cassava frogskin disease in leaves (A), presence of lips in root (B) and (C), and reduced root bulking (D).

Figure 20. Symptoms of Witches' Broom disease in cassava (Photo: B. Pineda)

Figure 21. Plants with exaggerated bud proliferation; shoot proliferation and/or unusually rachitic branches growing from single stake; and shoots with short internodes and small leaves that show no deformation or chlorosis were observed inVietnam. (Photo: J.F. Mejia)

Figure 22. Disease symptoms observed on cassava plants in the Philippines.

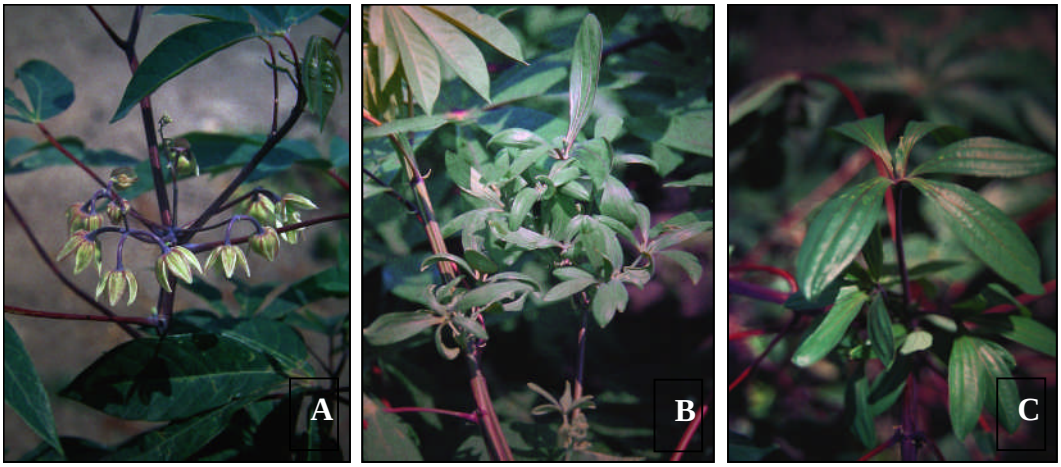


Figura 23. Symptoms of Antholysis in cassava. A, Healthy flower; B and C,Virescence and Phyllody. (Photos: B. Pineda)

Figure 24. Cassava mosaic disease (CMD) in Burundi (left) and Tanzania (right). (Photos: R. Howeler)

Figure 25. Symptoms of cassava brown streak disease (CBSD) on leaves (left) in Tanzania and roots (right) in Uganda (Photos: R. Howeler)

Figure 26. Cassava common mosaic disease (CsCMD) attacking leaves. (Photo: Maritza Cuervo.)

Figure 27. Mottled symptoms induced by the cassava virus X (CsVX). (Photo: Maritza Cuervo.)

CHAPTER 12. R.H. Howeler. Diagnosis of Nutritional Problems in Cassava

Photo 1. N deficiency on left; small plants but no clear symptoms

Photo 2. N deficiency; some varieties show general chlorosis of leaves

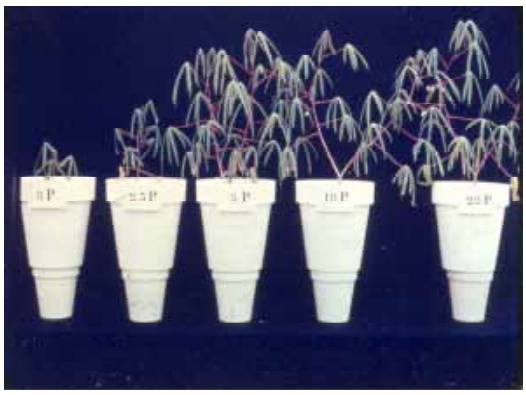


Photo 3. P deficient plants on left are small and spindly, and may have some yellow lower leaves

Photo 4. P check plot in front. Plants are small with some yellow lower leaves

Photo 5. K response in nutrient solution

Photo 6. K deficient plants in front. Upper internodes are short and leaves are chlorotic

Photo 7. K check plot in front. Plants have a prostrate growth habit with short internodes

Photo 8. K deficiency. Plants are highly branched and have a prostrate growth habit

Photo 9. Ca deficiency in nutrient solution. Edges and tips of upper leaves curl down

Photo 10. Ca deficiency affects the growth of young shoots and roots

Photo 11. Mg deficiency is characterized by intervenal chlorosis of lower leaves

Photo 12. S-deficiency results in a slight chlorosis of upper leaves, similar to N deficiency

Photo 13. S-check plot in field. Shorter plants with some chlorosis of upper leaves

Photo 14. B deficiency symptoms in nutrient solution. Seldom seen in the field

Photo 15. Speckling of leaves in middle part of the plant may be due to B deficiency

Photo 16. Cu deficiency is found mainly in peat soils

Photo 17. Cu check plot on right. Upper leaves are chlorotic and tips turn down

Photo 18. Response to Fe in nutrient solution

Photo 19. Fe deficiency in the field. Uniform chlorosis of upper leaves with little leaf deformation

Photo 20. Response to Mn in nutrient solution

Photo 21. Mn deficiency in the field. Intervenal chlorosis of leaves in the middle and lower part of the plant

Photo 22. Zn deficiency. Intervenal speckles on lower leaves

Photo 23. Narrow leaf lobes pointing outward due to Zn deficiency

CHAPTER 13. R.H. Howeler. Conducting Cassava Experiments

Figure 1. Response of 3-month old cassava to various levels of Mg using the programmed nutrient addition technique.

Figure 2. Flowing nutrient solution cultures to study the P response of cassava and other crops.

CHAPTER 19. R.H. Howeler. Importance of Mycorrhiza for P Absorption

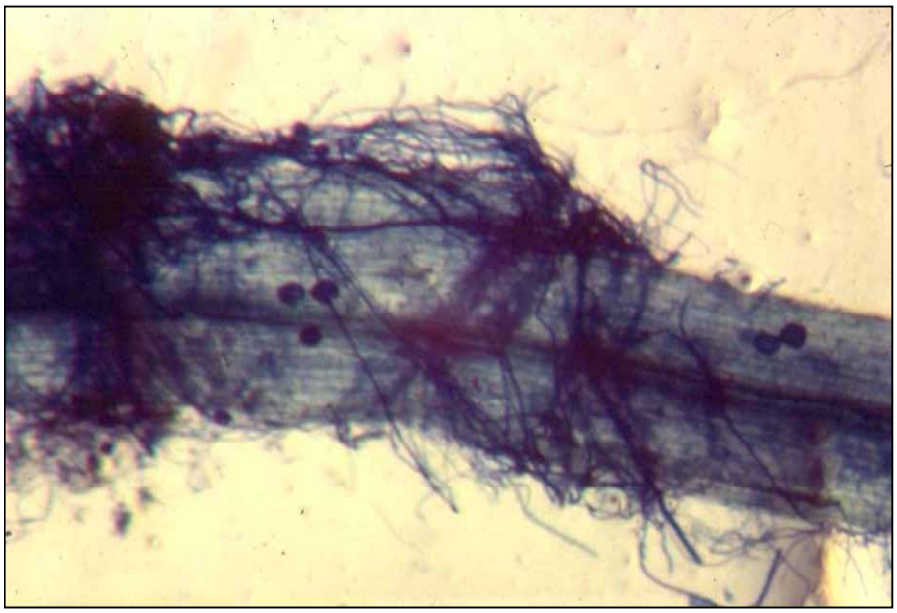


Photo 1. Fibrous root of cassava with vesicles in the root cortex and hyphae covering the roots in flowing nutrient solution culture.

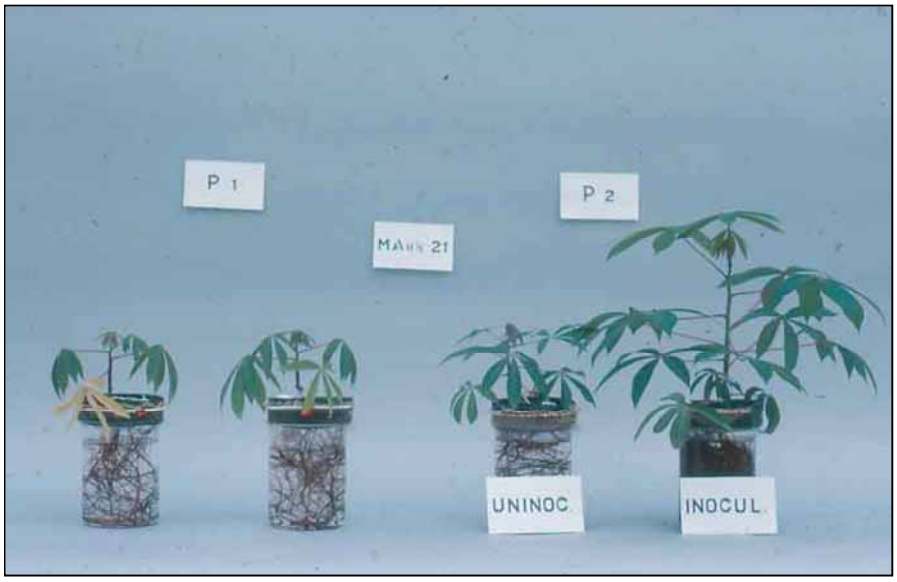


Photo 2. Growth response of cassava, MAus 21, to mycorrhizal inoculation (plants on right) when grown at 0.1 (P1) and 1 (P2) µM P in flowing nutrient solution culture.

Photo 3. Growth response to five levels of applied P, ranging from 0 to 3.2 t P/ha, in a sterilized Quilichao soil, without (top) and with (bottom) mycorrhizal inoculation.

Photo 4. Growth of cassava, cv. CM 91-3, grown in soil sterilized with methyl bromide in CIAT-Quilichao; on right plants inoculated with mycorrhiza, and on left without inoculation.

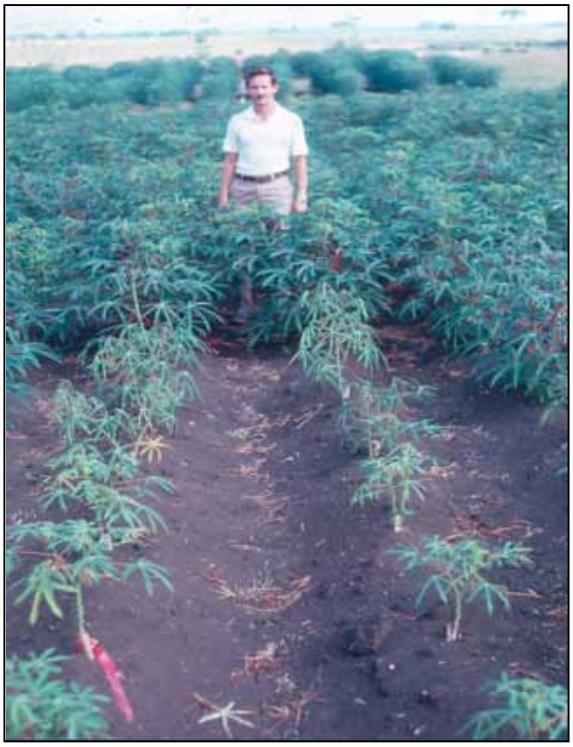


Photo 5. Growth of non-inoculated cassava, cv. MCol 638, in methyl bromide sterilized soil in front, and in unsterilized soil in back at 2 1/2 months after planting. Note symptoms of severe P deficiency of plants growing in the sterilized soil.

CHAPTER 20. R.H. Howeler. Soil Erosion Control

Photo 1. Soil loss by erosion can be very serious in sandy soils without any aggregate stability, even on very gentle slopes

Photo 2. On steep slopes soil loss by erosion can be very high

Photo 3. Once farmers see how much soil they lose due to erosion, they are likely to adopt soil conservation measures

Photo 4. Fertilizer application (in back) is one of the most effective ways to reduce runoff and erosion

Photo 5. Contour hedgerows of vetiver grass (in back) are very effective in reducing soil loss by erosion

Photo 6. Contour hedgerows of vetiver grass provide in-situ mulch and trap eroded soil sediments to form natural terraces

Photo 8. After seeing the effectiveness of vetiver grass hedgerows, farmers in Thailand planted 145 km of hedgerows

Photo 7. After ten years the hedgerows of Tephrosia candida and vetiver grass had formed 1 m high terrace risers

Photo 9. Water accumulating in natural drainage channels can wash out all the top soil

Photo 10. Gullies can be repaired by placing sand bags across the gully. These should be anchored in place with bamboo sticks

Photo 11. Once sediments collect behind the sandbags, vetiver plants can be planted in this wet soil to slow down the water flow

Photo 12. After one year an 80 cm high terrace riser had formed behind the sand bag and vetiver barrier

Farmer Participatory Research

Photo 1. In Vietnam cassava is often planted on very steep slopes where erosion can be a serious problem

Photo 2. In Thailand cassava is generally planted on gentle slopes but erosion can still be very serious

Photo 3. Researchers and extensionists conduct a Rapid Rural Appraisal in selected villages to learn about farmers' problems and opportunities

Photo 4. Researchers may also discuss problems and possible solutions with farmers in the field

Photo 5. Farmers prioritize their problems and possible solutions through participatory diagnosis techniques

Photo 6. Farmers may visit demonstration plots to evaluate several options that might help to solve some of their problems

Photo 7. Farmers in Vietnam discuss and evaluate several options to reduce erosion.

Phots 8. Farmers conduct simple erosion control trials on their own fields to compare some selected options

Photo 9. FPR erosion control trial with contour hedgerows of vetiver grass and Paspalum atratum

Photo 10. Once farmers see in their FPR erosion trials how some simple practices can markedly reduce soil loss by erosion, they want to adopt these practices on their production fields.

Photo 11. During a field day at time of harvest farmers visit all FPR trials and evaluate all treatments

Photo 12. After visiting all the FPR trials in the village, farmers and researchers discuss the results and select the best varieties and the most promising practices

Farmer-to-Farmer Extension

Photo 13. During cross visits or field days farmers conducting FPR trials show the visiting farmers their trials and the results obtained

Photo 14. Farmers also show how they have adopted the planting of contour hedgerows of vetiver grass to reduce erosion

Photo 15 . A participating farmer is being interviewed for TV during a farmer field day in Vietnam

Photo 16 . In Thailand many FPR trials were conducted by community-based self-help groups called "Cassava Development Villages"

Photo 17. During FPR training courses two key farmers of each village and their local extension agents learned how to help other farmers in their village conduct FPR trials

Photo 18 . After conducting FPR trials this farmer adopted a new high-yielding variety, intercropped with peanut, applied the right type of fertilizers and planted contour hedgerows of Tephrosia candida. His cassava yields increased from 4-5 t/ha in 1998 to 27 t/ha in 2000.

CHAPTER 22. T.M. Aye. Cassava Agronomy

Photo 1. Cassava planting by hoe in Laos

Photo 2. Land preparation by plowing with water buffalo

Photo 3. Land preparation by plowing with hand tractor in Thailand

Photo 4. Land preparation with ridges for vertical planting in Thailand

Photo 5. Land preparation in furrows for horizontal planting in Cambodia

Photo 6. Land preparation with mounds for cassava planting in Myanmar

Photo 7. Land preparation and planting cassava on mounds in India

Photo 8. Horizontal planting of cassava stakes

Photo 9. Inclined planting of cassava stakes

Photo 10. Vertical planting of cassava stakes

Photo 11. Simple cassava harvesting tool

Photo 12. Tractor-mounted cassava harvesting tool

Photo 1. Cassava intercropped with soybean in South Vietnam

Photo 2. Cassava intercropped with peanut in North Vietnam

Photo 3. Cassava intercropped with watermelon in Guangxi, China

Photo 4. Harvest of intercropped maize in Guangxi, China

Photo 5. In Indonesia cassava is often intercropped with upland rice and maize

Photo 6. Harvest of intercropped rice and maize in Yogyakarta, Indonesia

Photo 7. After the rice and maize are harvested farmers plant peanut between cassava rows

Photo 8. In East Java, farmers mainly intercrop cassava with maize

Photo 9. Cassava interplanted in a young rubber plantation in Cambodia

Photo 10. Cassava intercropped with soybean in Guangxi, China

Photo 11. Cassava intercropped with three rows of mungbean in Vientiane, Lao PDR

Photo 12. Harvest of intercropped peanut in North Vietnam.

CHAPTER 25. J. Buitrago. Fresh and Ensiled Cassava Roots and Foliage for Swine and Ruminants

CASSAVA CHIPPER (CLAYUCA, 2007)

CHIPPING FRESH CASSAVA (CLAYUCA, 2007)

FRESH CASSAVA CHIPS (CLAYUCA, 2007)

ENSILED CASSAVA CHIPS (CLAYUCA, 2007)

PIG FEEDERS FOR FRESH CASSAVA
(PERSONAL, 2006)

CALVES CONSUMING FRESH CASSAVA
(CLAYUCA, 2007)

CASSAVA ROOTS SILAGE IN SMALL POLYETHYLENE BAGS *(CLAYUCA, 2008)*

CASSAVA SILAGE IN BIG BAGS *(CLAYUCA, 2008)*

CASSAVA FOLIAGE SILAGE (CLAYUCA, 2008)

CHAPTER 26. J. Buitrago. Dry Cassava Roots and Foliage Meal for Poultry, Swine and Ruminants

SOLAR DRYING OF CASSAVA CHIPS *(CLAYUCA, 2006)*

DRYING TRAYS FOR CASSAVA CHIPS *(CLAYUCA, 2006)*

INDUSTRIAL DRYING OF CASSAVA CHIPS *(CLAYUCA, 2005)*

DRIED CASSAVA CHIPS *(CLAYUCA, 2006)*

DRIED CASSAVA FLOUR
(CLAYUCA, 2006)

CHOPPED CASSAVA FRESH FOLIAGE *(CLAYUCA, 2006)*

DRIED CASSAVA FOLIAGE FLOUR *(CLAYUCA, 2006)*

CHAPTER 27. J. Buitrago. Recent Developments with Dried Cassava Root and Foliage Meal for Poultry and Swine

CASSAVA ROOT FLOUR (CLAYUCA, 2006)

FULLFAT SOYBEANS (PERSONAL, 2005)

SOYBEAN TOASTER (PERSONAL, 2005*)*

SOYBEAN EXTRUDER (PERSONAL, 2005)

CHAPTER 28. B. Ospina *et al*. Use of Cassava for Small-scale Ethanol Production with Value-added By-Products

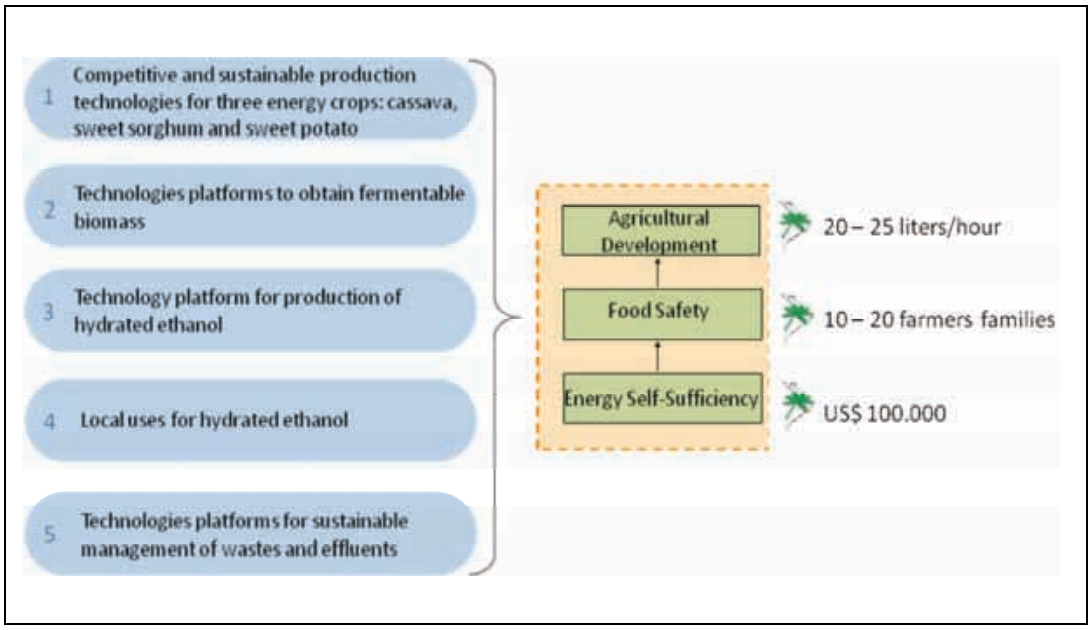


Figure 1. Technological components of the Rural Social Biorefineries (RUSBI) approach.

a) Hydrated ethanol processing plant.

b) Cassava/sweet potato flour milling and refining plant.

c) Effluents treatment plant.

Figure 2. Equipment included in a Rural Social Biorefinery (RUSBI).

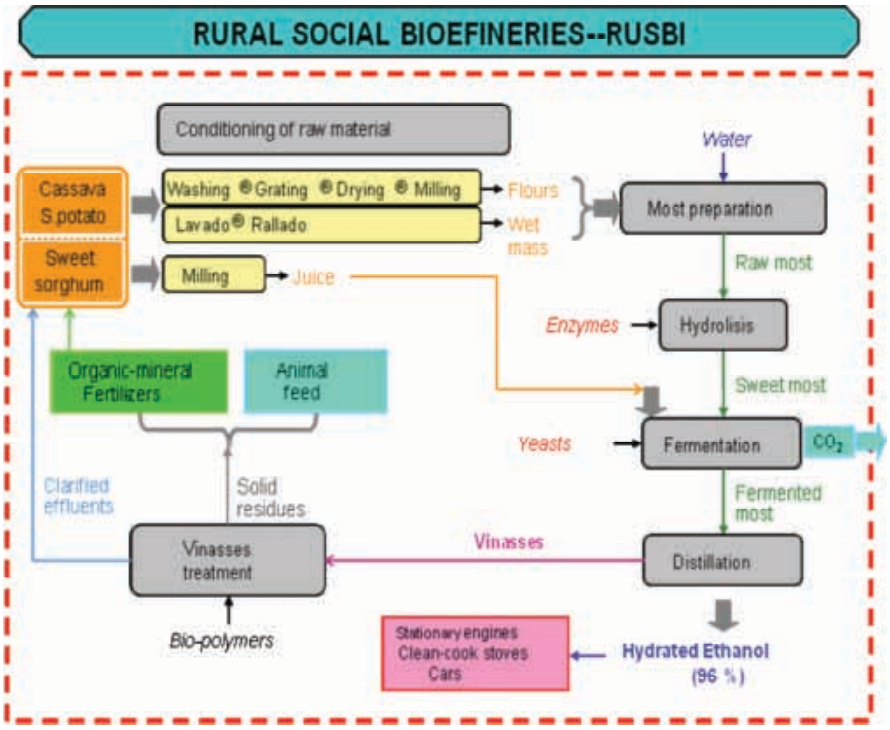


Figure 3. General scheme of the RUSBI approach.

Clean cook stove

Bio-electricity

Kit for flex-cars

Kit for flex-cars

Figure 4. Uses of hydrated ethanol.

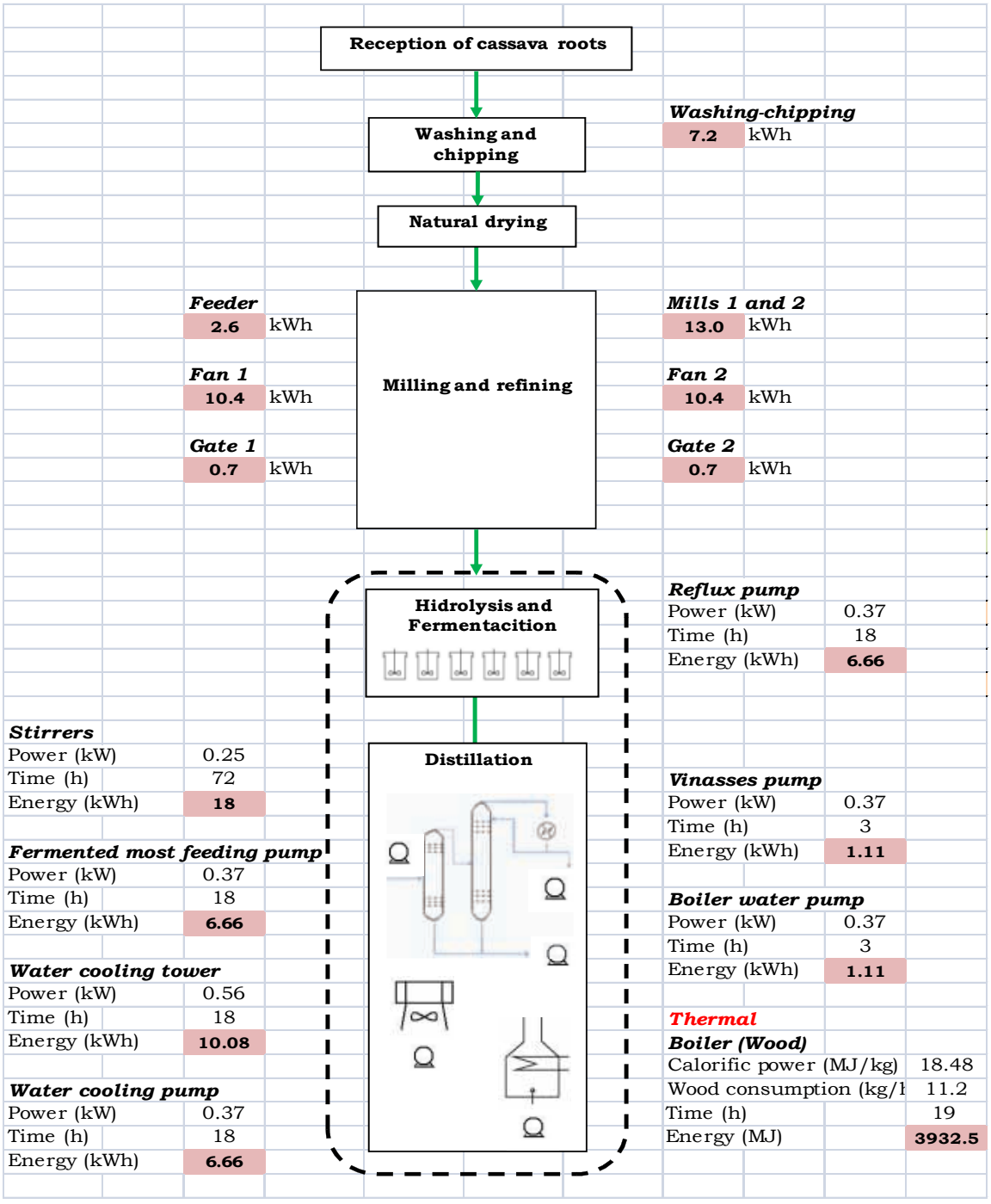


Figure 5. Energy balance for the production of hydrated ethanol bio-refinery from cassava flour. CLAYUCA Pilot plant.

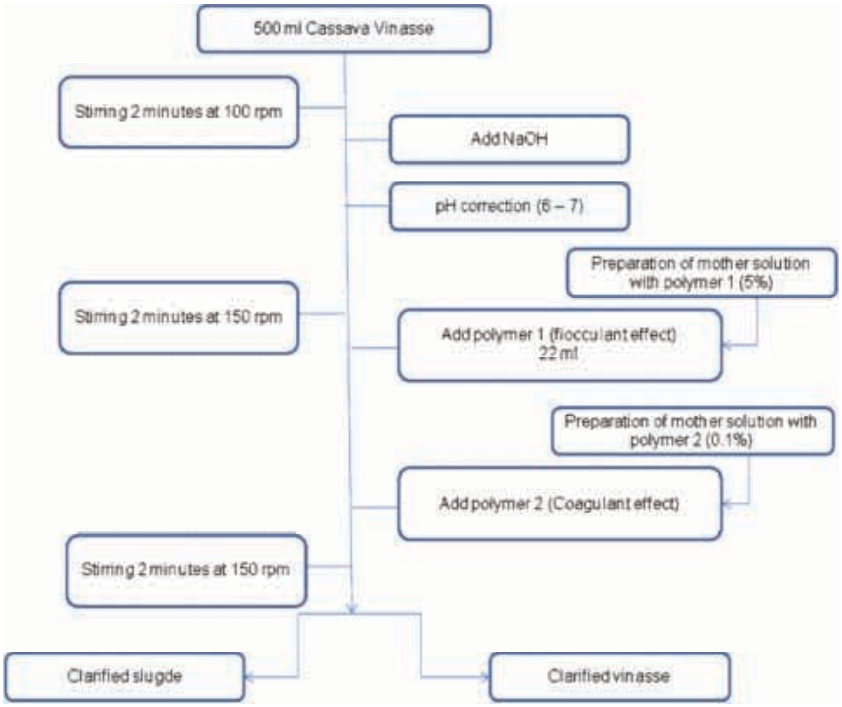


Figure 6. Flocculation and coagulation process for cassava vinasses.

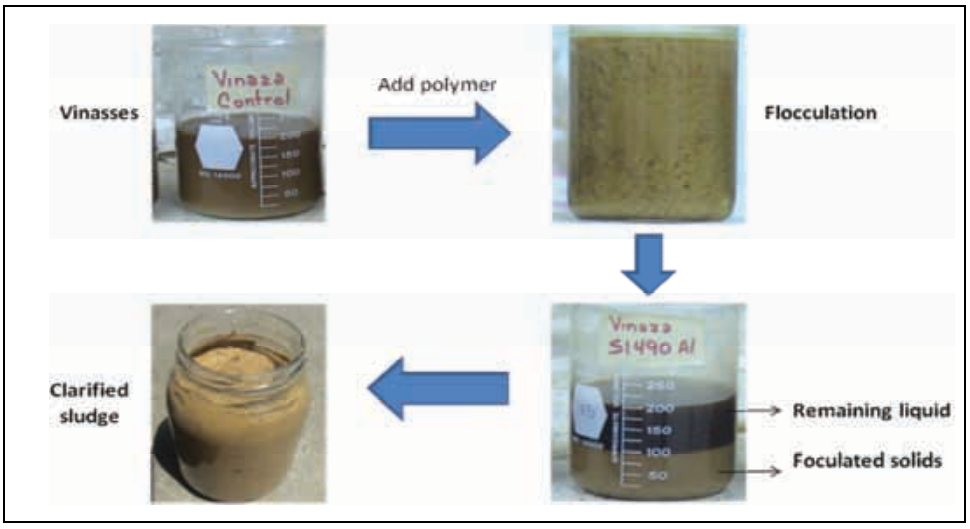


Figure 7. Scheme of the steps followed in the RUSBI approach to convert cassava vinasses into clarified vinasses and clarified sludge.

Figure 8. Consumption of a nutritional block made with co-products and effluents from sugarcane-based ethanol processing.

Figure 11. Experimental animals and grazing plots.

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