DOMESTICATION OF MULTIPURPOSE TROPICAL PLANTS, WITH PARTICULAR REFERENCE TO *Irvingia gabonensis* Baill., *Ricinodendron heudelotii* (Baill) Pierre et Pax and *Gnetum africanum* Welw.

by

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DECLARATION

I certify that this thesis has been composed by myself from the results of my own work, except where otherwise stated, and no part of it has been presented for a higher degree.

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DEDICATION

This work is dedicated to my late father
Papa Petro Nsoh SHIEMBO,
who first taught me how to tap Raphia **palmwine**
and my wife
Esther Bi SHIEMBO and our children
Tse Emmanuel and Siri Cybil SHIEMBO.

ABSTRACT

The tropical forests of Cameroon contain a wealth of multi-purpose plants from which a range of goods and services is derived, mostly by rural populations. The forests are currently under the pressure of exploitation for timber and other forms of economic development. To counter the effects of deforestation, this study was aimed at the domestication of three species for use in farming systems by rural people. These species, *Ricinodendron heudelotii*, *Irvingia gabonensis* and *Gnetum africanum*, were selected on the basis of their economic importance in South-West Cameroon.

For research on vegetative propagation, six non-mist propagators were built in the Forestry Research Station at Kumba, Cameroon. Stockplants were derived from cuttings and seedlings obtained from selected trees. Thirteen experiments were completed investigating the effects of applied auxin, leaf area and rooting media on the rooting of leafy stem cuttings of each of the three species. Additional experiments investigated the effects of fertilizer addition, shading and pollard height on stockplants of *Ricinodendron heudelotii.*

Results indicated that optimal rooting percentages of *Ricinodendron heudelotii* cuttings were obtained when treated with 40 μg IBA, inserted in sawdust, and with a leaf area above 80 cm^2. Similarly, optimum treatments for rooting of the cuttings of *Irvingia gabonensis* were defined as 200 μg IBA, inserted in sawdust, and with a leaf area above 80 cm^2, and those for *Gnetum africanum* were 250 μg IBA, inserted in sawdust, and with a leaf area above 80 cm^2. In the nursery stump height experiment, rooting of *Ricinodendron heudelotii* was found to decrease with increasing height, with an optimum height of 0.3 m. Higher rooting percentages of this species were recorded from fertilized stockplants, and from non-shaded stockplants, in additional nursery experiments. In a field experiment testing a wider range of pollard heights, rooting was again found to decline with increasing pollard height; highest rooting was obtained in the 1.5 m treatment. However, cuttings were successfully rooted from 10 m tall stockplants, which may enable reproductively mature cuttings to be successfully rooted using this approach.

These results indicate that these three species may be successfully propagated vegetatively using low technology methods, enabling domestication to proceed using clonal approaches. The implications of the results for the general understanding of the rooting process in tropical trees, and for the development of practical protocols for propagation on a larger scale, are discussed.

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CHAPTER 1

1. INTRODUCTION

1.1.0 The World's Tropical Rainforests

The world's tropical rainforests lie in a belt centred on the equator and extending about 23.5° north and south to the Tropics of Cancer and Capricorn, covering an area which was estimated in 1976 to be equivalent to that of the USA (Grainger, 1980). These forests constitute thirty percent of all the forests in the world (Grainger, 1980; US Interagency Task Force, 1980), and have been reported to cover about 6-7% of the Earth's land area (Ramakrishnan, 1992; Poore and Sayer, 1992; Whitmore, 1992; Smith *et al.*, 1992).

Tropical rainforests occur in three regions. About half occur in America where they are found in three principal areas, of which the largest lies in the Amazon and Orinoco basin. The second principal region is the South Eastern tropics centred on the Malay archipelago. Indonesia occupies most of this region and is second to Brazil in the area of rainforest it possesses. The third region, which is the smallest of the three, is in Africa, where it is centred on the Zaire basin, reaching to the Atlantic ocean at its western extremity, with an outlier in East Africa (Grainger, 1980; US Task Force, 1980; Gradwohl and Greenberg, 1988; Whitmore, 1992)

Many types of tropical forests have been described. Grainger (1980) stated that tropical moist forests comprise two general types known as tropical rainforest and tropical moist deciduous forest. In contrast, the US Task Force (1980) divided the tropical forests into three major types: (i) thorn woodland which is very dry forest,

(ii) moist forest with 1-2 dry seasons in the year and (iii) wet rainforest with little or no dry season. Recently, Whitmore (1992) identified and described six forest formations based on vegetation structure and physiognomy. These tropical forest formations are:

(i) Tropical lowland evergreen rainforest with a complex structure, and a large number of gregarious tree species, and no regular annual dry season.

(ii) Tropical semi-evergreen rainforest, which includes both evergreen and deciduous trees in an intimate mixture. Deciduous trees may comprise up to one-third of the canopy trees, though all are not necessarily leafless at the same time. The number of species is high, but less so than in evergreen rainforest, and the formation is marked by a regular annual period of drought, and it occurs in lower Amazon, most of the African rain forest block, including the whole Zaire basin, middle America, South China and to a very little extent in Southeast Asia.

(iii) Montane rainforests, which are of different structure and physiognomy and which vary with the type and height of the mountain. On the highest peaks, upper montane rain forest is replaced by a shorter more gnarled formation with smaller leaves (nanophylls) known as subalpine forest. This forest formation is found on the Andes, on the Caribbean islands, and in Africa where it occurs in Cameroon and at the eastern fringe of the Zaire basin.

(iv) Heath forest, which is a one-storey lowland tropical forest formation formed by saplings and small poles, which is dense and difficult to penetrate. This forest formation is found in the upper reaches of Rio Negro and Rio Orinoco in South America, and in Kalimantan, Sarawak, and Brunei in Borneo where they are called kerangas. They also occur in Brazil where they are known as campina, campinarana, caatinga Amazonica, or campina ropestre. The other principal occurrences are small areas in Malaysia, and the coastal sands in

Africa in Gabon, Cameroon, and Ivory Coast.

(v) Peat swamp forest, which is a lowland forest formation characterised by six different concentric forest types on peat domes. This lowland forest formation occurs in Sumatra, Malaysia, Borneo, New Guinea and in the Americas (including the pegass swamps of Guyana), and to a very limited extent in Africa.

(vi) Freshwater swamp forest, which is a formation in lowland forest made up of a diverse assemblage of forest types flooded by river water, with soils richer in plant nutrients than peat swamp forest. This forest formation occurs in the Amazonian freshwater swamp areas known as igapo, in the alluvial plains of Asia, and in the Zaire basin where one-third is occupied by swamp forests.

On a climatic basis, Whitmore (1992) stated that constant high temperature is a characteristic of tropical climates, with mean temperatures of the coldest month being 18°C and above. The U. S. Interagency Task Force (1980) pointed out that the tropical rainforests are associated with an annual rainfall of 4000 mm although there exist very wet areas with annual rainfall exceeding 4000 mm, for example, parts of Cameroon in West Africa.

1.2.0 General importance of tropical forests

It is now believed that about 40-50% of the world's plant and animal species occur in tropical rainforests (Myers, 1980; Whitmore, 1992; Poore and Sayer, 1992) the total species number of tropical forests has been estimated at two million species by Caufield (1982). Whitmore (1992) estimated that of the total of approximately two hundred and fifty thousand species of flowering plants in the world, about two-thirds occur in the tropics.

Tropical rainforests contain a wealth of natural resources that offer utilitarian benefits to human society in various ways (Myers, 1980). For example, forests moderate the climate both locally and globally by influencing the composition and the heat retaining capacity of the atmosphere and the heat and water exchange characteristics of the earth's surface. The tropical rain forests absorb incident solar radiation resulting in a low albedo which has a favourable influence on the hydrological cycle of tropical lands. They re-evaporate moisture deposited in rain, and much of this is condensed and deposited in rain elsewhere (Salati, 1987; Myers, 1979; Whitmore, 1992). Forests also protect catchment areas and ensure an adequate quality and steady flow of fresh water. They control erosion and sedimentation and are especially important where these affect downstream undertakings that depend on water for transportation, irrigation, agriculture and fisheries, and recreation (Nwoboshi, 1982; Poore and Sayer, 1992; Smith *et al.*, 1992). For example, Curry-Lindahl (1972) reported that production of Paddy rice and other irrigated crops in the tropics was especially dependent on forest-regulated water supply.

Tropical rainforests are also known to provide a vast range of foods, medicines, oils, dyes, resins, spices, latexes, tannin, dyes, ornamental plants, wildlife, fuelwood and source materials for pesticides and other kinds of industrial goods (Gentry and Dodson, 1987; Myers, 1988; Abbiw, 1990; Prance, 1994). These often play a significant part in rural economies (U.S.Interagency Task Force, 1980; Myers, 1979; Poore and Sayer, 1992; Whitmore, 1992; Smith *et al.*, 1992). In addition to this, they provide industrial products in the form of building materials, notably rattan, bamboos, smallwood and fibres (de Beer and Mcdermott, 1989). This is particularly true for Cameroon (Shiembo, 1986) and Ghana (Falconer, 1991), where most village houses of peasant farmers are constructed with these basic materials obtained mostly from the natural forests.

Rainforests are regarded by many people as a source of high quality timber and wood products for domestic and foreign markets. In many cases, this is very important for the generation of export earnings. Indeed, the demand for tropical hardwoods for furniture is growing in both the developing and developed worlds (Poore and Sayer, 1992; Sharma *et al.*, 1992).

Rainforests also play a part in the cultural heritage of the people in the countries where they occur. Poore and Sayer (1992) noted that forests contribute to the folklore and tradition of the people and often profoundly influence local culture. They often reflect past human use and may protect historical features. These authors further pointed out, for example, that in an inaugural speech of the IUCN General Assembly in Kinshasa in 1975, the Zairian head of state referred to the forest national park of Zaire as the "cathedrals" of his country. In Cameroon, many people believe that the forests harbour ancestral gods. Consequently, many big trees are worshipped and looked upon as sacred and capable of protecting human life. Different plants are regarded sacred in different localities. The "Boma tree" (*Ceiba pentandra* and *Bombax buonopozense*), for example, is believed in some villages in the South West Province of Cameroon to host good and evil spirits that are kind to well-behaved people and harm those who behave badly. For this reason, they are protected on farmland. In Mankon village, in Bamenda in the North West Province of Cameroon, it is believed that during the yearly worship of the ancestral gods, the "Fon tree" (*Piptadeniastrum africanum*) announces the death of important people in the clan.

Tropical rainforests are the Earth's main raw material base for drug yielding plants (Myers, 1979; Falconer, 1991), and provide innumerable species that are a source of drugs for both local and external use (Longman and Jeník, 1987; Whitmore, 1992). Some Amerindians of Amazona, for example, know of 750 plant species with medicinal properties (Myers, 1979). At least seventy percent of the 3000 species of plants that are known to possess anti-cancer properties exist in the tropics, where the huge stock of tropical forest plants still to be investigated could well supply many more in the future. Myers (1979) further pointed out that it was from Amazonian tribesmen who used *curare* (a muscle relaxant) on their arrow tips as a hunting poison that Western surgeons learned of the substance's potential for human operations. It is also reported by Hedberg (1982-3) that details of over 150 plants used in traditional medicine have been recorded in the tropical forest of North-East Tanzania.

The folklore of Africa, like that of other continents, abounds in medicinal plants. In Ghana, more than 800 woody plants have been found to have medicinal properties (Abbiw, 1990). In addition to this, 20000 plants from Ethiopia and Kenya have been screened between 1960 and 1976 by the medicinal plant resources laboratory of the US Agriculture Research Services for chemical compounds that show promise in the fight against cancer (Abbiw, 1990). For the same purpose, the National Cancer Institute has so far screened 29000 species of plants from other tropical countries (Grainger, 1980). The seeds of the Australian rain forest legume, the Moreton Bay chestnut (*Castanospermum australe*) have recently been discovered to contain a drug that might help combat AIDS (Whitmore, 1992). It has also been reported by the US National Cancer Institute (Miller, 1993) that in the course of work in the Korup National Park in Cameroon a vine (*Ancistrocladus korupensis*) was found, which appears to inhibit HIV through an identified alkaloid called michellamine B. There are high hopes that this drug will help in the treatment of AIDS. This illustrates the potential of the tropical rainforests to meet new human requirements as they arise.

1.3.0 Destruction of tropical rainforests

Because of their importance as a source of timber and other non-timber products, the tropical rainforests of the world are being heavily exploited. Rates of deforestation continue to increase (Table 1.1) posing a grave threat to environmental and economic stability at global and regional levels. This is one of the major environmental problems now facing mankind (Gradwohl and Greenberg, 1988; Myers, 1989). The problem is further aggravated by growing populations, which counteract the maintenance of a degree of forest cover which can fully satisfy human requirements for raw materials as well as environmental protection (Whitmore, 1992). It has even been suggested that unless governments individually and collectively take action, by the end of the first quarter of the next century the world's tropical forests will be nothing but scattered remnants (US Interagency Task Force, 1980), if not completely eliminated (Myers, 1980).

Threatened elimination of the tropical rainforests is no exaggeration since the forests of the world are being degraded at an alarming rate. Gradwohl and Greenberg (1988), for example, pointed out that deforestation is now proceeding at an annual rate of at least one percent, up to two percent if areas under selective logging are included, and that, extrapolating from these figures, twenty to forty hectares are disappearing every minute. They further pointed out that in some countries the situation is even worse. In the West African countries of the Ivory Coast and Nigeria, for example, the rate of deforestation is as high as five to six percent of the total land area a year. However, the rate of deforestation varies from country to country. In some countries, such as Indonesia, Brazil, Madagascar, and the West Indies, there are areas showing explosive rates of deforestation, and in certain parts of the Amazon, deforestation rates appear to be following an exponential, rather than linear trajectory. Currently, the worldwide rate of deforestation is on the increase (Gradwohl and Greenberg, 1988), and it is feared that all tropical countries with forests are losing these complex and valuable ecosystems. As a result, plant and animal genetic and other resources whose value we may never ever know will also disappear (Smith *et al.*, 1992).

Table 1.1: Rates of deforestation and tree planting in some tropical countries in West and Central Africa (Source: World Resources Institute, 1992-93).

	Extent of natural forests and woodland (kha in 1980)			Annual deforestation (kha in 1981-85)				Plantations (kha)
	Closed forest	Open forest	Other woodland	Closed forest		Total forest		
				(kha)	(%)	(kha)	(%)	
Benin	47	3,820	6,832	1	2.6	67	1.7	0
Cote d'Ivoire	4,458	5,376	15,390	290	6.5	510	5.2	6
Ghana	1,817	6,975	9,480	22	1.3	72	0.8	2
Guinea	2,050	8,600	9,000	36	1.8	86	0.8	0
Liberaia	2,000	40	5,640	46	2.3	46	2.3	2
Nigeria	5,950	8,800	49,450	300	5	400	2.7	26
S. Leone	740	1,315	4,278	6	0.8	6	0.3	0
Togo	304	1,380	3,720	2	0.7	12	0.7	0
West Africa	17,267	36,306	104,690	703	4.1	1,199	22	36
Cameroon	17,920	7,700	15,600	80	0.4	110	0.4	1
Central Africa	70,395	11,915	71,575	307	0.2	575	0.2	3
Gabon	20,500	75	19	1,500	0.1	0.1	0.1	19

1.4.0 Status of non-timber forest products in tropical forests

Many tropical countries possess a very rich and diverse resource base of forest products, which have both immediate and long-term potential for economic, social and cultural use. These resources are traditionally divided into two main groups referred to as timber and non-timber resources (Mackay, 1941 and Peters *et al.*, 1989). The timber resources have been classified as major forest produce and the non-timber as minor produce (Sarin, 1981; Abbiw, 1990). This distinction of major and minor products has directed attention to the major forest produce in matters of organizational arrangements in the form of structure, systems, training of manpower, planning procedures, and many other considerations, to the near exclusion of the minor forest produce (Sarin, 1981).

The non-timber resources are further divided into wood and non-wood resources of the forest and come from plant and non-plant sources. Plants from which these products are obtained are referred to as multipurpose species if they provide more than one product. In this study, multipurpose trees will be defined as the plants from which minor forest products are tapped, and the terms minor forest products and non-timber products will be used interchangeably. Although often classed as minor, the non-timber tropical forest products have tremendous contributions to make to rural and national economic development. It is not unusual to find that some locally available minor forest products are at least equal to timber products in importance (Said, 1963; Poulsen, 1982). The actual market benefits of timber are often small relative to those of non-timber resources to the rural inhabitants (Peters, Gentry and Mendelsohn, 1989). In commercial forests, non-timber products make up about 50% of the total economic value of the total forest resources, and in protected forests this rises to about 90% (Cherkasov, 1988). In the forests of Thailand, some of the non-timber products are much more valuable than timber per unit forest area (Phenglai and Khamsai, 1985). In the tropical rainforests in general, the number of different minor forest products exploited by millions of people is incredibly large, and if their value is properly assessed, may even be found to outweigh that of timber (de Beer and Mcdermott, 1989).

1.4.1 Types and uses of non-timber forest products

(i) Food

There are many different kinds of food gathered from forests, ranging from termite larvae to leaves and mushrooms (Falconer, 1988). Some forest food is made up of non-woody plants such as *Talinum triangularae* which is collected and prepared as a vegetables, sauce, condiment and flavouring (de Beer and Mcdermott, 1989). In the forest zone of Cameroon where this study was carried out, the leaves of *Gnetum africanum* are collected as a vegetable which is eaten nationwide and exported in large quantities to neighbouring Nigeria and Gabon. Fruits, seeds, kernels and nuts of some tree species in the natural forests of Cameroon are collected as food, snacks, spices, condiments, soups, oils and food supplements (Personal observation).

Forest fruits commonly collected in Cameroon include bush mango, both the sweet and bitter varieties; bitter kola; Njabe seeds; and those of *Ricinodendron heudelotii* (Njangsa); *Tetrapleura tetraptera* (Sekeseke); *Trychosypha accuminata* (Change blood or bush mbongmbong); *Kola* spp.; *Xylopia aethiopica*, and *Canarium schweinfurthii* (Bush plum) to name just a few. These fruits, nuts and kernels are put into various uses. They fetch immediate cash income for the collectors. Seeds and kernels are seasoned by drying, and local techniques have been developed to store some nuts fresh all the year round. These seeds, kernels and nuts are sold on a daily basis in urban and rural markets throughout the year. They are commodities for both internal as well as external trade, including export to neighbouring countries, principally Nigeria, Gabon and Chad.

Other items of food from tropical forests are roots, vines, tubers, rhizomes, and corms which provide starch, a typical example being the wild yams (*Diascorea* spp) (de Beer and Mcdermott, 1989). The tuberous roots of ***Gnetum africanum*** are also eaten for their starch by Pygmies in some areas of Cameroon (Bahuchet, 1990). Minor forest products collected from tropical forests for food include mushrooms, mammals, birds, reptiles, snails, amphibians, fish, forest insects and caterpillars. These are used as a source of proteins by the forest dwellers of Southeastern Asia (de Beer and Mcdermott, 1989) and by those in Cameroon (personal observation). Some wild palms are tapped in the West African tropical forest areas for their sap, which makes very good palm wine. In the region from which this author comes, ***Raphia*** sap commonly called "mimbo", "matango" in the eastern region and "matoto" in the coastal area of the southwestern region commands a unique significance in all areas of social life. It is the liquor of central importance on all social occasions such as marriages, birthday parties, traditional dances, death celebrations, meetings of farming groups and most importantly, it is the main source of income to the ordinary man in this region. It was from the sale of ***Raphia*** palm wine that this author was educated up to the first academic degree level.

Most forest plants supply food to people indirectly through the provision of feed for domestic animals. In some localities in Southeastern Asia, sago pith and the refuse left from processing is fed to pigs (Ruddle *et al.,* 1978), and in most villages in southern Cameroon, wild cassava, potatoes (both tubers and fresh leaves) and wild guava are collected and given to livestock (personal observation). Fodder is not usually collected for livestock such as pigs and fowls; rather they are allowed to forage freely in the forests.

(ii) Medicine

Of all the categories of non-timber forest products, the sources of forest-derived medicines are the most difficult to characterize (de Beer and Mcdermott, 1989). This is true for several reasons, among which is the great diversity of wild species involved, and the degree of overlap with every other class of non-timber forest product. For example, in the forests of Thailand, Indonesia and Malaysia, the traditional pharmacopoeia includes the fruits of *Averrhoa blimbi* L., edible oils of "illipe nut", or *Shorea* spp., resins of *Styrax benzoin*, tannins of *Uncaria gambir* and ornamentals such as *Cassia* spp. The same distinction made between food and medicine as is observed in the West does not apply here. Many foods such as vitamin-rich forest herbs are considered to have health-giving properties and many foods and beverages may be taken as tonics. Certain plants and fungi are known to have hallucinogenic, narcotic or stimulant effects and are ingested for ritual and recreational purposes.

Traditional medicine employs a minimum of 4.5% of the indigenous Southeast Asian flora, and this constitutes a pharmacological supply of at least 560-900 species from the lowland rain forests of Malaya, Borneo and New Guinea alone (Jacob, 1982). The World Health Organization compiled a collection of over 21,000 plant names that were reported to have medicinal uses world-wide (Jacob, 1982), and Perry (1980) listed over 6,000 species used by traditional healers in East and South east Asia.

In general, one or more parts of many plant species are used in combination to provide medicines. The commonly used plant parts are leaves, roots, fruits, nuts, barks, latexes, seeds, stems, grains and vines. The list is long and no attempt is made here to be exhaustive. Different combinations of these items are prepared, depending on the malady, as tinctures, infusions, concoctions, decoctions, and extracts (Abbiw, 1990). In a rural setting in the tropics, these traditional medicines are used in treating all health cases. Those collecting tropical plant materials to screen for medicinal purposes have always been guided by "native doctors", as they are called in most parts of West Africa.

(iii) Industrial non-timber products

The tropical forests of the world also offer minor forest products which are used as raw materials for the production of economically important end products in rural craft centres. The canes of rattan palms, for example, are used in the construction of frameworks for furniture such as chairs, baskets, tables, etc., and split cane is woven into mats and various handicrafts. Like rattan, bamboo is a culturally important mainstay of traditional technology in villages across Southeast Asia (de Beer and Mcdermott, 1989). Bamboos are used for the construction of houses, fences and furniture in most parts of Cameroon (personal observation). Another non-timber forest product of industrial importance is fibre. Fibres obtained from forest vegetation are of great importance to African communities (Poulsen, 1982; Shiembo, 1986). They are used for making baskets, mats, rope, furniture and in house construction and high quality fibres are exported overseas for the manufacture of brushes and wickerwork. In recent times, an industry has developed in most West African countries involving the use of both *Raphia* and oil palm wine in the production of dry gin. It used to be known as illicit gin because its production was not legalised. It has now been legalised in Nigeria and Cameroon. The gin is produced using local technology and raw materials. As a result, it is cheap and the value added at each stage of production makes the industry quite profitable (personal observation).

1.5.0 Species under study

Originally, three fruit trees, namely *Irvingia gabonensis*, *Ricinodendron heudelotii* and *Garcinia kola* were selected for this study, based on the fact that the products from these species are among the most valuable non-timber forest products in Cameroon. It soon became clear, however, that *Garcinia kola* is not only slow-growing, but it is also very difficult to produce sprout shoots from coppice stumps. As a result, this species was replaced with a wild vegetable which is highly priced and is currently one of the most economically important products, fetching immediate cash income for the local population all over the rainforest areas of Cameroon. This vegetable is collected from the wild populations of a vine of a rare climber represented by two species in Africa, of the Gnetaceae family. These species are *Gnetum africanum* and *Gnetum buchholzianum*. The vines of the former species, which was identified by a staff member of the national herbarium in Yaoundé, form part of the present study.

1.6 Objectives of the Study

I. The general and long-term objectives of the work of which this study forms an important part are:

(a) to domesticate multipurpose plant species of the moist forest of Cameroon by:

  i) developing vegetative propagation techniques,
  ii) determining the biological, ecological, physiological and pathological factors affecting the domestication process,
  iii) selecting superior trees for propagation for future use in agroforestry farming systems.

(b) to develop an agroforestry farming system using domesticated material of the selected species.

II. The specific research objectives of this study reported in this thesis are to:

(a) select superior phenotypes from which

  i) to collect seeds for seedling stockplants, and
  ii) to coppice as a source of juvenile shoots for vegetative propagation;

(b) examine the effects of various factors affecting rooting of cuttings obtained from coppice shoots or seedlings. This work forms the basis of Chapters 3, 4 and 5.

(c) pollard mature trees at various heights and evaluate sprout quantity and quality, and relate this to the rooting ability of cuttings taken from them;

(d) determine the best management requirements of shoots from pollarded mature trees, to obtain good rooting ability. This will involve experimental studies to compare the rooting ability of these shoots with those from juvenile coppice shoots, and the effects of shading;

(e) identify and propagate superior phenotypes known by villagers in the study area. Selection will be done on the basis of fruit yield and flavour;

(f) establish a clonal base of these species at the Forestry Research Station at Kumba. It is expected that this will be used by research workers for future studies involving many other useful multipurpose plants which could not be investigated for this thesis.

1.7.0 Characteristics of experimental species

(i) *Irvingia gabonensis* (Sweet bush mango)

Irvingia gabonensis Baill is a tree of the moist forest which belongs to the family Irvingiaceae, which is in turn made up of three genera, all the species being trees (Keay *et al.*, 1964). The other genera are *Klainedoxa* and *Desbordesia*, which do not bear fruits with edible pulp. This species is unrelated to the semi domesticated commercial mango (*Mangifera indica*). *Irvingia gabonensis* is a large tree with evergreen foliage, in some cases reaching 35 m in height and 120 cm in diameter.

The crown has a more or less conical shape which makes it stand out distinctly in the forest (Plate 1.1). These features make it easier for fruit collectors to locate the trees. It is evergreen, flowers from November to March, and fruits from June to July (personal observation) (Figure 1.1), with a mean fruit diameter of 6.3 ± 0.4 cm, although Aina (1990) reported that in Nigeria the fruits are available between the months of July and October. In Cameroon, there are two species (or varieties), namely *I. gabonensis*, the sweet bush mango which fruits in the rainy season, and *I. excelsa*, the bitter bush mango (Okafor, 1975) which fruits in the dry season (Figure 1.1). The kernels of *I. excelsa* have greater drawability (sliminess) than those of *I. gabonensis* (Okafor, 1975). *I. excelsa* has been found to grow mostly in the south western part of the country (mainly in Mamfe: a town in Manyu Division) near the Nigerian border.

General products and production techniques

The fruit pulp of *I. gabonensis* is eaten and the kernels of both species are eaten in soups and as cakes from which oil is obtained by baking the cake over fire. The pulp of the sweet mango constitutes about one-third of the whole fruit, while the kernel of both the sweet and bitter species make up only about one-tenth of the whole fruit. The fruits are processed for kernels in the bush under the mother trees in order to cut down transportation costs. The powdered kernels are used for medicinal purposes in the treatment of wounds and the oil obtained from the kernels is used for soap-making (Abbiw, 1990). In Ghana, the seeds of this tree are used as cattle-cake (Abbiw, 1990). The wood is hard, heavy and has fine grain (Thikakul, 1985), and is sawn for general purpose timber in many localities in Cameroon. In Ghana, the wood is used as house posts, for ship-building and for carving domestic utensils. On farms where they are protected for their fruits, the bush mango trees provide shade for food and cash crops as coffee and cacao. They are also known to restore soil fertility by releasing nutrients from the leaf litter (Songwe, 1984).

Plate 1.1: Sweet bush mango (*Irvingia gabonensis*) tree protected on farmland to serve as a shade tree and as a source of fruits and kernels for the farmer.

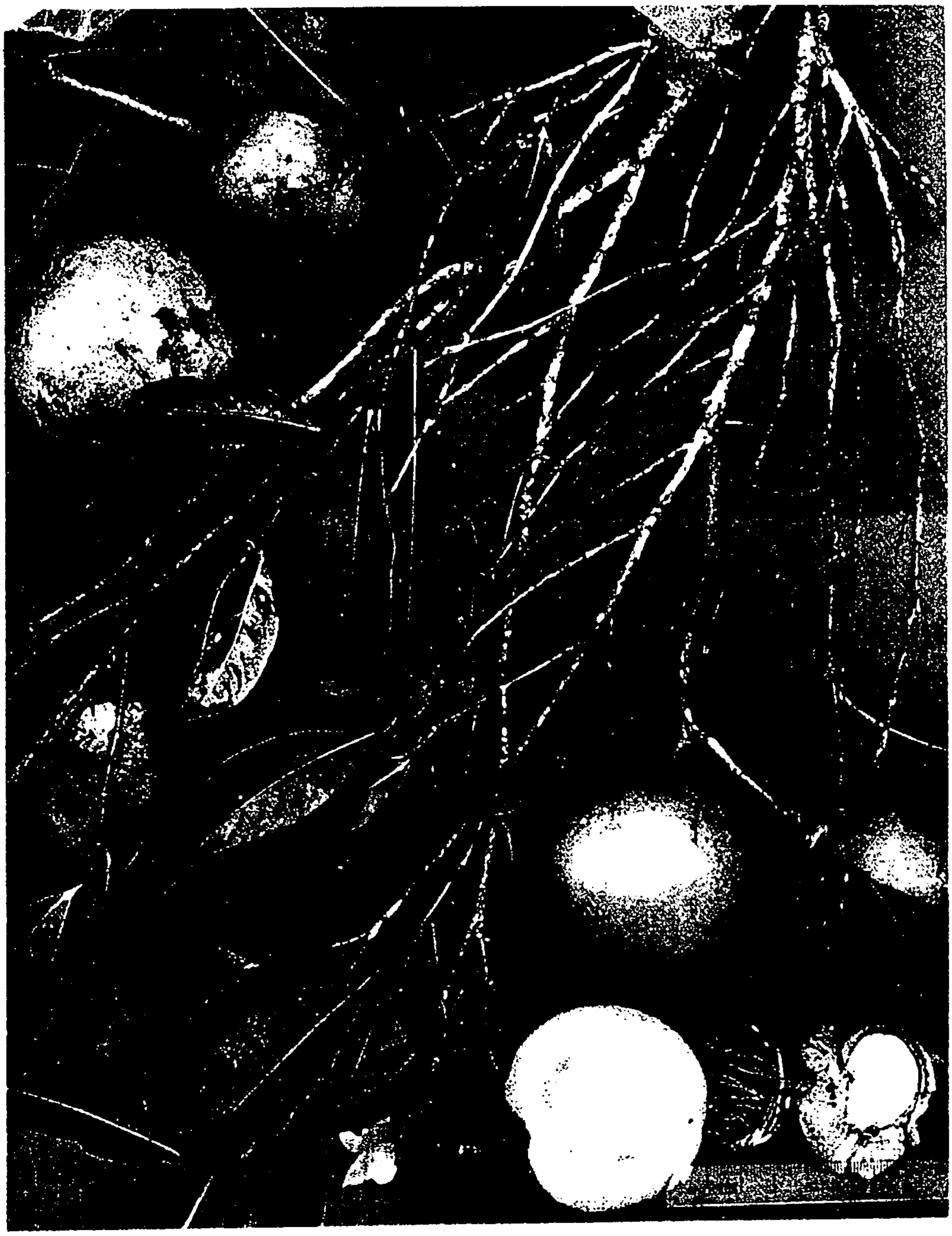


Figure 1.1: Branches of *Irvingia gabonensis* laden with fruits; and cross sections of some of the fruits (Source: Hladik, M and Hladik, A., 1990).

The collection of the fruits and kernels of *Irvingia gabonensis* takes place in natural forests as well as on farms. However, in the Mamfe area, some farmers have planted a few trees from where they are now able to collect fruits. In natural forests, the collectors locate the trees in flower during the dry season and check before fruit drop that no-one else is collecting from the same tree. This is easier in reserves than it is in communal forests. When the fruits start to drop, the collectors select fruits with very sweet and thick pulp which are taken back to the villages where some are eaten and the others are taken to the village or town markets for sale. Most of the fruits are processed for their kernels using two main techniques. One of these methods involves placing a sharp cutlass carefully into the pulp along the suture of the tough endocarps and hitting the fruits, one after the other, against a piece of wood to separate the two cotyledons which are then extracted from the endocarps using a small knife. The second method involves packing the fruits together in heaps for the pulp to rot away. The tough endocarps (seeds) are then cracked using a small baton thereby exposing the kernels. This extraction exercise takes place under the parent trees in the forests or on farms (Plate 1.2) The kernels so processed are taken home, dried and used for food or taken to the market for sale. The kernels can either be dried in the sun or in barns above the fireplace.

The silviculture of Irvingia gabonensis.

When the fruit of *Irvingia gabonensis* drop, they take less than one month to lose their pulp completely. Profuse germination immediately follows the loss of pulp. The resulting wildings almost all perish within the following six weeks except in situations where they happen to fall on fertile soil, and where there is ample light. The wilding growth is quickly arrested after they have produced four to five leaves. At this stage, the roots and root hairs become highly lignified and brittle. If they are collected for potting at this point, they tend to die. High germination percentages may be obtained by picking up ripe fruit, depulping them, then partially drying and sowing the seeds. The seeds of this species lose their viability in a matter of a week

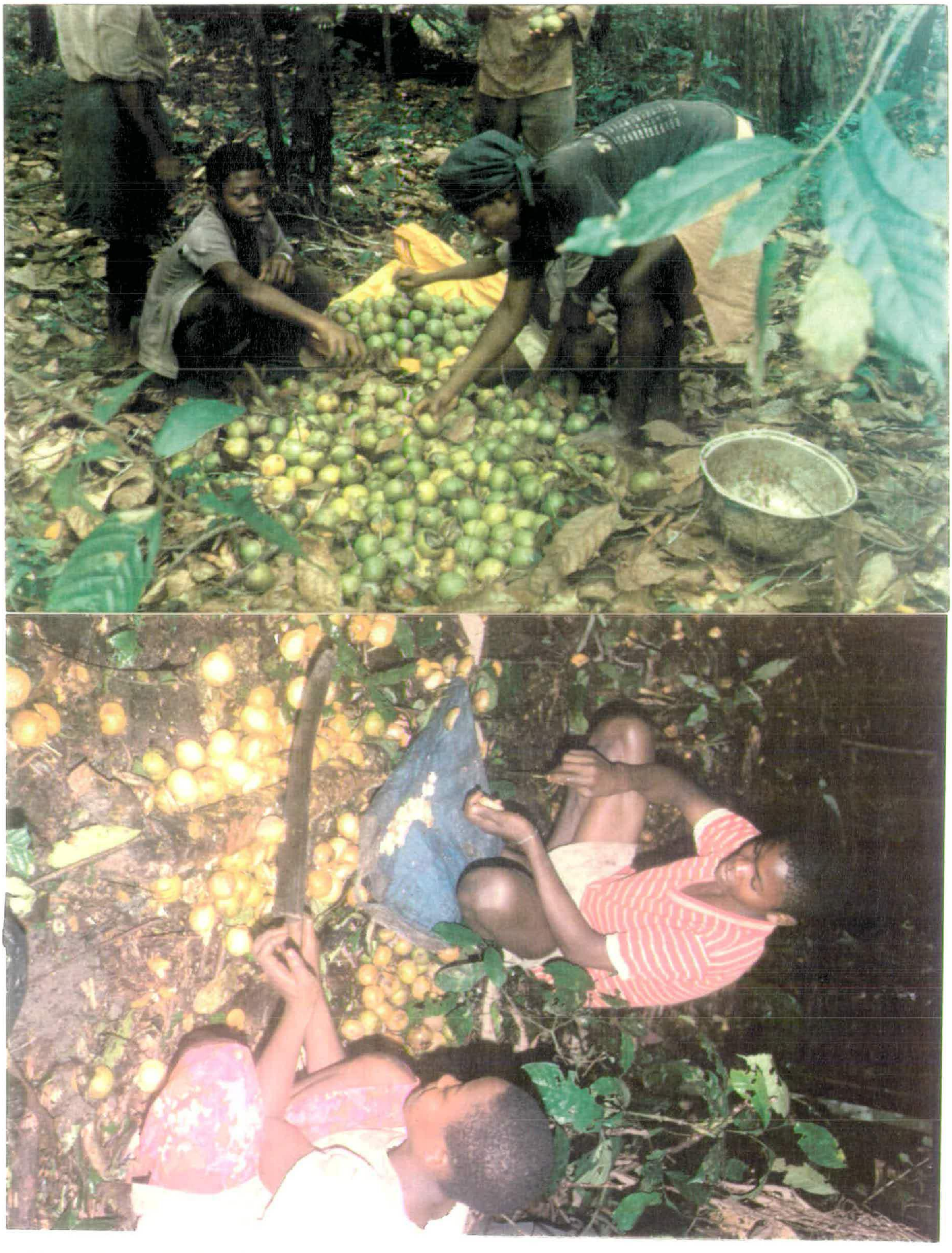


Table 1.2: Fruit collectors at work, collecting, gathering in heaps and cracking the fruits of *Irvingia gabonensis* (sweet bush mango) in the forest in Kumba, Cameroon.

or two when stored under ambient conditions. The period of viability may be extended if stored in a cold room. After the seedling stage, the material can be planted out in the field and maintained for a future crop.

(ii) *Ricinodendron heudelotii* (Baill) Pierre ex Pax

Ricinodendron heudelotii (Baill) Pierre ex Pax is a large deciduous forest tree which belongs to the Euphorbiaceae. The members of this family are generally characterised by the presence of rubbery white latex in different parts of the plant, by the swelling at the apex of the petiole and by the presence of glands on various parts of the leaf blade (Keay *et al.* 1964; Letouzey, 1986; Thikakul, 1985). *Ricinodendron heudelotii* is particularly characteristic of secondary forest, and is identifiable by its compound-digitate leaf with 3 to 5 leaflets. The bark is scaly and brown with a granular reddish slash (Thikakul, 1985). In the Southern Bakundu forest reserve, *Ricinodendron heudelotii* occurs as very large trees, reaching 45 m in height and 150 cm in diameter. The bole is often straight, but is sometimes sinuous or twisted with an enlarged channelled base. If the bole is hit with the back of a cutlass, it produces a characteristic hollow sound (personal observation).

Unlike *Irvingia gabonensis* which is monoecious, *R. heudelotii* is dioecious with the female trees producing fruits from March to November. In general, both male and female trees flower in March at the beginning of the rainy season which corresponds with their flush period. During the dry season, the trees shed their leaves completely and only flush at the beginning of the rainy season. The fruits are bi- or tri-lobed (3.8 ± 0.2 cm mean fruit diameter) containing 2 or 3 stones in which the seeds are embedded (Figure 1.2). The seeds in the bi-lobed fruits are generally larger than those from tri-lobed fruits. The seeds, which are the edible parts, constitute only one-third of the stone on a dry mass basis. Processing of the seeds is less costly if completed near where the mother trees are located. It is reported by seed collectors that the seeds from the bi-lobed fruits have a better taste than those from tri-lobed fruits. The habitat of this species in secondary forest makes it an ideal multipurpose tree for planting on farms, the practice of which has been very rare since farmers cannot tell, at a seedling stage, which tree is female or male. During site preparation at the start of a new farm, the existing trees of this species are protected to eventually serve as fruit producing tees as well as shade trees.

General products and production techniques

The wood of *R. heudelotii* is very soft and light. Consequently, people rarely use it for general construction purposes. It is, however, used for making light house implements such as wooden dishes, long drums, wooden spoons and carvings such as masks and dolls. The stony seeds are used as rattlers in gourds to produce musical sounds (Personal observation). In Ghana, the ash obtained by burning the wood of this tree is used as a substitute for table salt and for making soap, the bark infusion is used as a medication against abortion, the root or root-bark decoction is taken in the form of a beverage as an enema and the bark is pounded, warmed and applied to treat elephantiasis (Abbiw, 1990). The fact that the ash is used as table salt suggests that it contains sodium in quantity. The bark has also been found to treat leprosy in Zaire (Bokemo, 1984). When stumped, a lot of sprout shoots with broad leaves are produced resulting in high biomass production, which is very good for mulching, thereby forming a good source of organic manure. When protected on farms, it provides shade. The oil obtained from the seeds of this tree is used for making soft soap and for varnish (Abbiw, In Cameroon, the seeds are a very valuable ingredient for soups, they are complements to many dishes and fetch immediate cash income among the farming population in the areas where they thrive. The seeds are called "njangsanga" in the region where this study was carried out. They form an important article of trade among the farming population where they are sold in all the markets throughout the year.

Figure 1.2: *Ricinodendron heudelotii* flowering branch. A = male flower, B = stamens, C = stamen, D = female flower, E = ovary, F = cross-section of ovary and G = fruit (Source: Hutchinson, Dalziel and Keay, 1954).

During the flowering and fruiting season, prospection for trees bearing fruits is made by the *R. heudelotii* fruit collectors. These visits are generally repeated to assess the quantity of fruits on each tree so as to reinforce their rights to collect from the trees in question. During fruit drop, the collectors go to the different trees and gather the fruits in heaps and allow the pulp to rot away and free the stones in which the seeds are embedded (Plate 1.3). After the rotting operation, the stones are washed clean, carried home and boiled. In the course of boiling, the stones crack and fall apart to free the seeds which are now separated from the husks, washed and dried for consumption as well as for sale.

The silviculture of Ricinodendron heudelotii

After the pulp has rotted away, the stony nuts containing the seeds lie under the tree for more than two years if they do not germinate or are not carried away by rodents (Personal observation). The germination of the seeds of this species is very unpredictable and seems to be governed in the main by the decomposition of the stony endocarp. Many attempts have been made by this author to apply such germination pretreatment as scarification, partial burning under a thin layer of grass and giving the hard nuts alternate soaking in cold water and drying, to no avail. In their natural state, profuse germination takes place when the regrowth round the parent trees is cleared at the start of the first rains in the month of March. The wildings grow very luxuriantly and soon get attacked by Psyllids which curl the leaves and make it difficult for the seedlings to photosynthesise. The seedlings are soon smothered by natural regrowth. However, the seeds that are carried away from source germinate and grow among other species without problems.

At the start of the rainy season, there is mass germination of the seeds of *Ricinodendron heudelotii* under the parent female tree. The wildings can be collected for potting before the Psyllid attack. While in the nursery, insecticides can be used to ward off the Psyllids. In this way, the this species can be raised

Plate 1.3: The fruits of *Ricinodendron heudelotii* being gathered in a heap in a forest in Kumba, Cameroon.

successfully in the nursery, although the seeds are difficult to germinate and if the wildings are collected at the wrong time, they are infested with the Psyllids.

(iii) *Gnetum africanum* Welw.

The genus *Gnetum* belongs to the family Gnetaceae which is one of the three families that make up the order Gnetales (Dutta, 1979). *Gnetum* is related to conifers and other gymnosperms (Goulding, 1985). Many members of this genus are lianas which are either male with catkins of stamens, or females with catkins of ovules barely protected by an envelope (Letouzey, 1986). The genus *Gnetum* contains 40 species distributed in the equatorial regions of Africa, America and Asia. *G. buchholzianum* Engl. and *G. africanum* Welw. are edible plants commonly used in equatorial Africa (Ouabonzi *et al.*, 1983). These two species occur in the southwestern as well as southeastern parts of Cameroon and are common in forest edge habitat. The species that occurs in the study area is *G. africanum* (P. Mezili, National Herbarium, Yaoundé, Personal Communication, 1992). When a leaf of any of the two species is torn apart, white fibrous hairs are seen on the torn edges. The internode of the male spike of *G. africanum* is of the same diameter throughout its length while that of *G. buchholzianum* is much thinner at the base than it is nearer the apex (Hutchinson and Dalziel, 1954) (Figure 1.3).

General products and production techniques

Although both species are dioecious and pose a problem of sex determination, this causes no problems to cultivators because the organ of interest is the leaf and not the fruit. *Gnetum africanum* (known in Anglophone Cameroon as "eru", in Francophone Cameroon as "okok", in Nigeria as "okazi" and in Central African Republic, Zaire, Congo, and Gabon as "koko") is a vegetable collected from the wild and commonly used in the raw state as a salad, or cooked as the major vegetable of soups. The salad is often eaten as a mild laxative, and its high fibre content helps prevent

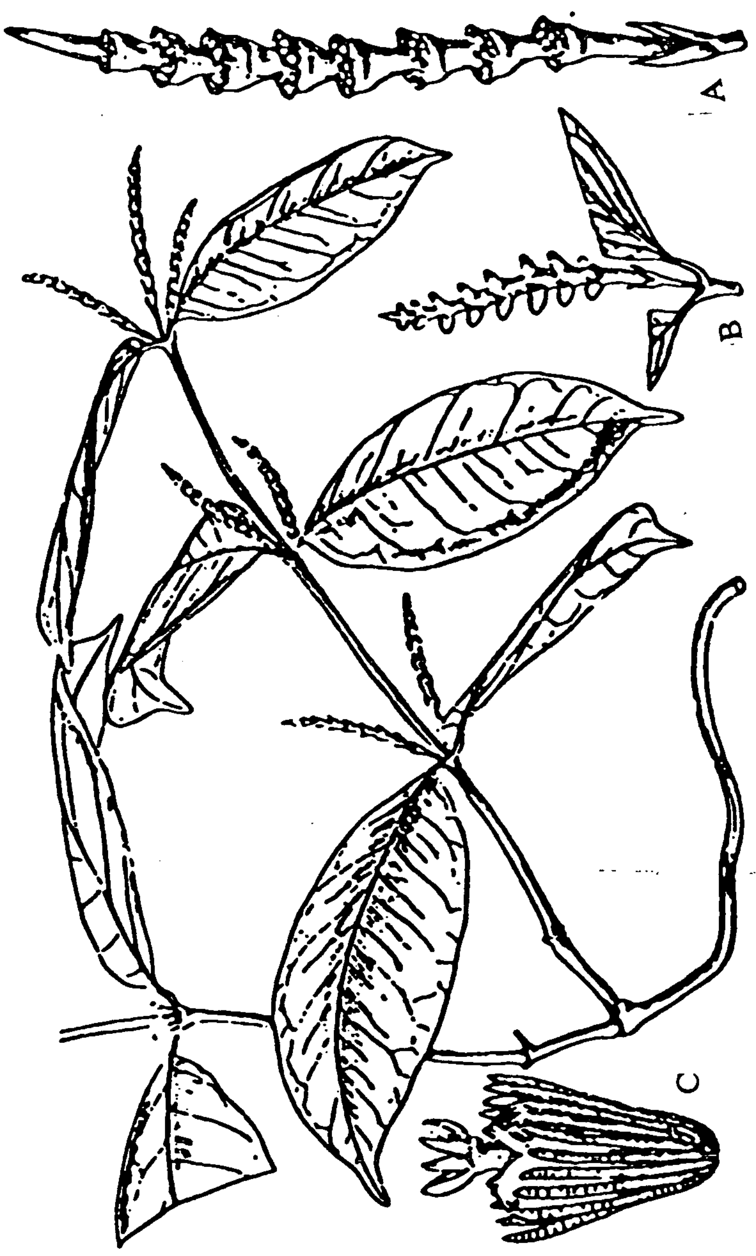


Figure 1.3: *Gnetum africanum*. A = male spike, B = female and C = female flower, (Source: Hutchinson, Dalziel and Keay, 1954).

constipation (Abani, 1988). The seeds are surrounded by red fleshy material that is attractive to fish, which act as seed disseminators in some areas (Goulding, 1985).

In Cameroon, eru is the most important wild vegetable collected from natural forests for local consumption, and local and external trade with neighbouring countries. The nutritive status of this vegetable is very high. The leaves of koko have a high protein content (about 30% of dry mass) and are used as a vegetable by all the ethnic groups of the rainforest area in the Congo Basin and the southern part of Central African Republic. Before they are boiled in water, the leaves are minced because of their fibrous texture (Bahuchet, 1990). They can be added to various dishes including meat and mushrooms, with the sauce made of fatty seeds or palm oil. The tuberous root of the climber is also edible but is used exclusively when yams or cassava tubers are not available. In the region where this study was carried out, the minced leaves of eru are boiled in very little water with ***Talinum triagulare*** (water leaf) or the leaves of ***Xanthosoma mafaffa*** (macabo) to make them soft and more palatable; a lot of palm oil and meat are added to "cooked eru". The main dish which is eaten nation-wide and sold in all business places by market women and young people is commonly called "water fufu and eru". The traders make a lot of money from the sale of water fufu and eru particularly in the South West Province of Cameroon.

Before the collectors of this vegetable go out into the primary or secondary forests for large scale collection, two or three days before the main market day are taken to prospect the likely areas where a heavy harvest can be carried out for commercial purposes. After locating such areas, the collectors go out in twos or threes, for safety, the day before the main market day of that locality to harvest the leaves of this climber. The harvest is done by pulling down the vines from the trees or shrubs which support them, and picking the leaves. The leaves are tied in bundles, each of which is measured by putting fingers round the leaves, and fastening them with ropes. The bundles are then kept in a cool place in readiness for conveyance to the market the next day. The collectors in the course of the harvest cut down young economically valuable trees. When the climber is also cut and pulled down from tree tops, the cut vine does not resprout and may cease growth completely.

The silviculture of Gnetum africanum

Gnetum africanum produces bright red fruits which contain seeds which when sown never germinate under nursery conditions. This author has made several attempts but has failed to germinate the seeds of this vine for the past three years. Some wildings have, however, been found germinating on the forest floor from where they have been collected, potted and tended. Their growth performance has so far been less than satisfactory. Apart from the poor growth performance, it is difficult to say whether the wildings will grow to produce the kind of leaves required. Broad-leaved and fast-growing vines were easily selected by this author in the field for research into vegetative propagation.

1.8.0 Importance of domestication of non-timber forest products

In agriculture and horticulture worldwide, herbaceous plants and a few woody species have been domesticated from the wild to provide improved "crop" plants with greater yield and higher quality products. Although terminology and definition regarding the incorporation of plants into human affairs vary considerably (Smith *et al.*, 1992), the term "crop" has been defined by Simmonds (1984) as a plant cultivated to yield a useful product or products, and the term "domestication" as human induced change in the genetics of population to conform to human desires and agroecosystems, culminating in the plant's loss of ability to survive in the natural ecosystem (Harlan, 1975 cited by Clement and Villachica, 1994). For a full domesticate to survive, human intervention is required. However, in the early stages of domestication, wild sources of a product and domesticated populations co-exist for a time, but economically, the crop soon ousts the wild sources, which are lost.

A few decades ago, timbers such as many pines, spruces, eucalyptus and teak were harvested from the wild; nowadays, wild stands and early domesticates co-exist and both are exploited.

Domesticates must have originally been selected for a number of desired characters (Simmonds, 1984; Libby, 1973). The desired characters that make them superior to their wild forms include, in the case of fruit trees, sweet fruits, large seeds, lack of toxins and high contents of desired constituents such as oils, alkaloids, aromatics, juices and latex (Rediske, 1974; Simmonds, 1984; Libby, 1973). Other considerations are good flavours in fruits, nuts and beverages, and small size and non-spininess in fruit trees (Simmonds, 1984), but large and straight stems, and superior wood quality in timber (Rediske, 1974). For example, by domesticating the oil palm, gains in oil yield have been impressive. Smith *et al.* (1992) reported that oil yields increased by approximately 23% as result of the first selection cycle from plantings in the later half of the nineteenth century. For much of the twentieth century, yield increases have averaged between 10 % and 15% per eight-to ten-year generation (Hardon *at al.*, 1987, cited by Smith *et al.*, 1992).

In general terms, the ideal domesticate may be disease resistant; have a high capacity to grow quickly thereby shortening the production and regeneration periods; possess a high degree of adaptability to a wide range of environmental conditions and a high degree of genetic uniformity and stability (Leakey *et al.*, 1982a; Libby, 1973; Rediske, 1974). In the tropics, most species currently exist in natural forests in their wild forms, although most of them may not be in their ecological zone of origin (Smith *et al.*, 1992) and only very few have been even partly domesticated. Coconut (*Cocos nucifera*), Guava (*Psidium guajava*), Rubber (*Hevea brasiliensis*), Pineapple (*Ananas comosus*), Oil palm (*Elaeis guineensis*), Avocado (*Persea species*), Orange (*Citrus* species) and Mango (*Mangifera indica*) and sago palms, some tannin producers (eg *Acacia*) and a range of other fruit plants, are in the position where the domesticates are fast replacing their wild ancestors as economic resources (Simmonds, 1984).

1.9.0 Techniques of domestication

Tropical forests have been considered by Smith *et al.* (1992) as rich hunting grounds for new plants to domesticate. Plants are domesticated to serve many purposes. Tropical plants may be domesticated to yield timber, fruits and many non-timber products from a broad range of plant families. Domestication is necessary as a means by which selection from the great genetic variation (both additive and non-additive) within species can be captured (Libby, 1973).

A number of approaches have been used in tree domestication both in the temperate zone and in the tropics. Before the domestication process commences, outstanding genotypes as expressed in the phenotypes are collected by intensive selection from among wild forms from a wide variety of ecological sources. Selection of superior genotypes can be accomplished in two major ways. Firstly, indigenous peoples who have had long intimate associations with wild and domesticated plants should be contacted (Libby, 1973; Smith *et al.*, 1992; Simmonds, 1984). In some cases, however, non-tribal people in rural areas, who have known about plant resources in the forests, can be consulted. Even recent settlers can help identify potentially useful plants, particularly if they have hunted or gathered forest products with old-time residents (Moran, 1981 cited by Smith *et al.*, 1992).

Secondly, selection could be from an established orchard or gene bank. A gene bank or orchard is a collection of outstanding genotypes (Libby, 1973; Ladipo *et al.*, 1994), gathered from one or more locations as grafts, seedlings, or cuttings. In seed orchards or gene banks, these outstanding genotypes are protected from many of the risks to which natural populations are exposed, such as loss to logging, fire (Ladipo *et al.*, 1994; Libby, 1973), or disease (Libby, 1973).

Breeding of industrial forest trees began in the 1950's with *Pinus* species (Barnes and Simons, 1994). However, the improvement of non-industrial trees is relatively new and involves many species (Simons *et al.*, 1994). The aim here is to capture, conserve and utilise the genetic resources of multipurpose non-timber trees in the tropics. To achieve this, breeding material must be got by sampling to avoid inbreeding depression, exploit heterosis, develop the potential for cloning, manage and use the genotype-environment interaction (Barnes and Simons, 1994). This involves provenance selection (Leakey, 1982a), where seeds are collected from a variety of locations throughout the natural range. In this case, once the initial breeding population is established, advanced selection of progeny with superior genotypes is carried out within the population. Progenies may be tested in one or more environments for evaluation, (Libby, 1973; Libby *et al.*, 1969). The number of trees to be evaluated is generally large, to generate a sufficient selection differential while maintaining a broad genetic base (Libby, 1973). To ease field evaluation and selection decisions, Libby (1973) recommended that the plantings should be made on appropriate forest sites. An objective of progeny testing may be to further test genotypes already established in seed orchards. However, the principal value of progeny tests is the validation of the selection of the parents (Libby, 1973).

There are shortcomings in applying these approaches to forest tree domestication, however. Individual forest trees have large sizes and long generation times (Leakey, 1987; Rediske, 1974; Libby, 1973). This affects both the length of time to reproductive maturity and that necessary to evaluate each succeeding generation (Libby, 1973). The phenotypical characteristics in demand are usually manifested only in the mature trees (Rediske, 1974). In addition, many of the candidate species for domestication have seed supply problems. These problems range from infrequent flowering, short periods of seed viability to damage by pests and pathogens (Leakey *et al.*, 1982b).

These problems have led to the development of other domestication techniques which include vegetative propagation. This technique has been widely and successfully used to domesticate crops in horticulture and has the additional advantage in that by producing genetically identical trees, it allows promising genotypes to be easily identified and selected. Vegetative propagation can be done by grafting or budding, marcotting and rooting leafy stem cuttings. In the present study, rooting leafy stem cuttings has been extended from timber trees to some of the multipurpose trees of the moist forest zone of Cameroon. One of the approaches envisaged in the implementation of this study involves the domestication of selected multipurpose plant species native to this region for eventual inclusion in agroforestry farming systems. For this domestication programme, vegetative propagation offers the best starting point as it allows the capture and selection of the genetic (additive and non-additive) variation present in individual plants. In addition, it allows the manipulation of these plants, some of which are naturally very tall, for use by local farmers in Cameroon.

Almost all the fruit trees planted in farmland in most parts of tropical Africa have been introduced from the neotropics or southeast Asia (Owen, 1973). All the conventional fruit species in Cameroon are exotic. Of the one hundred and fifty species of indigenous edible woody plants covering one hundred and three genera in forty-eight families occurring in Nigeria and Cameroon (Okafor, 1980), only *Dacryodes edulis*, *Cola acuminata* and *Cola nitida* have been cultivated in Cameroon (Personal observation). The rest are wild, and when any is encountered during bush clearing, they are protected by the farmer. The three species under study here are most commonly seen growing in the wild, although *Irvingia gabonensis* has recently been planted on private farms in Mamfe near the Nigerian border. Those who are engaged in the harvest of the produce of these species primarily obtain them from the natural forests. The density of these species in the natural forests is low (Okafor, 1980). The cost of the long distance search conducted by villagers to collect the fruits and vegetables of these species is generally high although the revenue realised from the sale of them is quite substantial. In the wild, *Gnetum* is highly threatened by the growing number of collectors, particularly at times such as the current economic recession.

The two fruit tree species in this study are not so threatened by fruit collectors because the fruits are collected from the ground after fruit drop. They are more threatened by the rapid degradation of the forest environment in which they occur. As in Amazonia (Prance, 1994), the extraction of many products from forests may lead to over-exploitation rather than the sustainable use of the resource. Nobody claims ownership of multipurpose species in natural forests, resulting in indiscriminate harvesting of the resource. Trees are better protected on farms where they are covered by ownership rights.

Not only are farmers happy to have these economically valuable species on their farms, they are interested in growing improved varieties. Such material may have the additional advantage of earlier fruiting and dwarf stature if reproductively mature material can be propagated vegetatively. The other problems that cloning these species will solve for farmers include their inability to recognize the species at the seedling stage and to know whether or not they are planting male or female trees. The farmers need planting materials to be available at the time they wish to plant. While this project was in progress, many farmers visited the propagation site to ask for planting material of the species. These people include women's groups such as the Catholic Women Association, the Reverend Sisters, Women Union of Primary School Teachers and Tombel Women Farmers' Group. The owner of Mukete Farms in Kumba also contacted the author offering to carry out the on-farm research component of the work on one of his farms.

It is hoped that by raising plants from cuttings obtained from mature trees and from sprouts obtained from pollarded trees some degree of dwarfing will be attained, thereby facilitating fruit collection. It is also expected that the fruiting ages of the plants so raised will be reduced. Aina (1990) stated that vegetative propagation (grafting) of the two varieties of *Irvingia* by the Forestry Commission of Nigeria has reduced the fruiting age from ten to about three years. Okafor (1978) found that trees of *Treculia africana* raised from stem cuttings produced fruits within three years and the trees were dwarfed to heights very close to the ground. Shiembo (unpublished data) also found that *Psidium guajava* pollards produced cuttings which flowered and fruited after one rainy season at ground level (Plate 1.4).

1.9.1 Vegetative propagation techniques

Vegetative propagation involves the multiplication of plant species using asexual techniques. This method of plant reproduction employs plant parts such as individual cells, tissues and organs to reproduce complete plants. This is possible as cell division continues to occur in the meristem during normal plant growth and development (Hartmann and Kester, 1983). This ability of plants, referred to as totipotency, is not shared by the animal kingdom (Pandey, 1981). Entire plants, for example, have been developed in aseptic cultures from individual cells of tobacco pith (Vasil and Hildebrandt, 1965) and carrot root (Steward *et al.*, 1963).

In recent times vegetative propagation has become a widely used method of raising timber, fruit and ornamental plants in most countries both in the temperate and tropical zones. Cloning by using reproductively mature plant material enables the exploitation of a single superior genotype, the elimination of the juvenile phase and a shortening of the time to reach fruit production stage (Hartmann and Kester, 1983; Leakey, 1987). The more general importance of vegetative propagation lies in the selection and multiplication of both additive and non-additive gene effects (Biondi and Thorpe, 1981) and in the potential for more rapid genetic gains by cloning as

Plate 1.4: Cloned guava (*Psidium guajava*) that flowered and fruited one rainy season after planting out.

compared with provenance selection (Leakey, 1989a). However, clonal propagation is not without its problems (Bonga, 1981). Some clones deteriorate in time due to accumulation of pathogens, particularly viruses, over a number of generations of vegetative reproduction (Shields and Bockheim, 1981; Bonga, 1982). For example, "Roter Stettiner", an apple clone which for a 500-600 year period was one of the most productive clones in central Europe, has become almost extinct because of gradually increasing degeneration problems due to accumulation of viruses (Passecker, F. cited by Bonga, 1981).

1.9.2 Cuttings

In propagation by cuttings, a portion of a stem, root, or leaf is cut from the parent plant and induced to form roots and/or shoots, thus producing a new independent plant which is genetically identical to the parent plant (Hartmann and Kester, 1983). Vegetative propagation techniques have been in use for a very long time. The Japanese, for example, used them with *Cryptomeria japonica* (Ono, 1972) hundreds of years before forest genetics was recognized, and similar techniques have been utilized for a long time with poplars (*Populus* spp.) and willows (*Salix* spp.). Chinese fir (*Cunninghamia lanceolata*) has been grown from rooted cuttings in China for several centuries (Minghe, 1988).

Rooting of stem cuttings is one of the most important means of vegetative propagation for mass multiplication (Uniyal, Prasad and Nautiyal, 1993). In Cameroon as elsewhere in West Africa, cassava (*Manihot esculenta*) is commonly propagated by stem (non-leafy) cuttings by the farming population. It is also common practice to see live fences delimiting farmland, building plots and roads planted with cuttings cut from ornamental plants and directly inserted into the ground without any special pretreatment.

1.9.3 Grafting and budding

Grafting involves the connecting of two pieces of living plant tissue together in such a manner that they will unite and subsequently grow and develop as one plant (Hartmann and Kester, 1983). One piece forms the upper part and is known as the scion and the lower part is termed the rootstock. The only difference between grafting and budding is that the graft is a shoot, while in budding, the scion is a bud. The rootstock can either be raised from seed, from a cutting or from layering. Wells (1955) stated that successful grafting requires:

(a) Compatibility of the scion and the rootstock such that the parent plant of the graft or scion must be related. They can be clones or varieties of the same species.

(b) Correct attachment of the scion to the rootstock in such a way that the cambium of the scion or graft is in direct contact with that of the rootstock.

(c) Enough care given to the graft until the union between stock and scion is complete.

Hartmann and Kester (1983) added that the grafting operation must be done at the time when both the rootstock and the graft should be physiologically healthy, and that immediately after the grafting operation, all cut surfaces should be protected from desiccation by covering the graft union with tape or grafting wax.

1.9.4 Air layering

Layering is a vegetative propagation method by which adventitious roots are induced to form on a stem while it is still attached to the parent plant. The rooted stem is detached to become a new plant growing with its own roots. Layering is a natural means of reproduction in species such as raspberries and trailing blackberries, or may be induced artificially (Hartmann and Kester, 1983). For layering to take place, the following conditions must be fulfilled (Hartmann and Kester, 1983):

(a) The stem remains attached to the parent plant during rooting and is continually supplied with water and minerals through the intact xylem.

(b) The stem is bent or wounded at the point where roots will be formed. This treatment depends very much on the position of the stem.

(c) Light is excluded from the rooting position by surrounding it with the rooting medium and holding it in place with a piece of polythene sheet or cloth.

1.9.5 Tissue culture

Tissue culture involves the production of plants from organs, tissues, or cells grown aseptically where the environment and nutrition can be rigorously controlled. The ability to grow plant tissues such as callus and cell suspensions, and various plant organs such as stems, flowers, roots, and embryos, has been utilized for decades as a research tool for geneticists, botanists, and plant pathologists (Hartmann and Kester, 1983). The biological principles of tissue and organ culture were first enunciated in 1902 by the German plant pathologist Haberlandt, and by 1934 tomato roots were grown continuously *in vitro* by supplying them with yeast extract (White, 1963). Since then, a large number of developments in the use of this technique have been made. These include the discovery of haploid plant formation from developing pollen grains (Nitsch and Nitsch, 1969), the isolation of plant protoplasts (Cocking, 1960), and the artificial (parasexual) hybridization of plant protoplasts in aseptic culture (Carlson *et al.*, 1972).

The application of tissue culture techniques to regeneration and commercial propagation of whole plants is a more recent development. It has become an important alternative to more conventional propagation procedures for a wide range of plant species. It is used as a technique for rapid mass propagation, development, maintenance, and distribution of specific pathogen-tested clones. Tissue culture systems have the potential for long distance shipment of propagation material, long-term storage of clonal material (Bajaj, 1979), and production of secondary products such as pharmaceutical in cell suspension systems (Dougall, 1979) as well as many applications in plant breeding (Brettell and Ingram, 1979). However, in forestry tissue culture has not yet found wide application, mainly because for many tree species cloned propagules still cannot be obtained from culture of tissues of mature plants (Bonga, 1981). However *in vitro* vegetative propagation of mature trees has, in a few cases, been achieved with explants other than those taken from reproductive structures. Shoot sections of sandalwood (Lakshmi Sita *et al.*, 1979) and terminal buds of mature eucalyptus (Gupta *et al.*, 1981) have regenerated new plantlets.

1.9.6 Conclusion

Vegetative propagation, as the only alternative to raising of plants from seeds, has many advantages. These include:

(a) Availability of planting material is ensured since it can be propagated at any time of the year. Generally, the plants can be raised more rapidly than seedlings and can be taken straight out to the field, avoiding a year in the nursery (Longman, 1976).

(b) Uniformity and improvements in planting stock quality are achieved more easily.

(c) Creation of tree gene banks is possible because selected clones can be planted in orchards for future propagation and/or research work. In this type of clonal orchard, genes can be recombined *via* controlled pollination (Biondi and Thorpe, 1981).

(d) Plants can be dwarfed by producing them vegetatively. For example, plants propagated with buds taken from the adult phase of the plant, invariably begin to flower at a younger age than the original seedling plant. In one example, grafted sugar maple ramets flowered in two years in the greenhouse and seven to nine years in the open, but comparable seedlings plants did not flower until nineteen to twenty-one years (Gabriel, 1979)

(e) Vegetative propagation enables genetic gains to be made in growth and product quality, using clonal selection techniques.

Cuttings were chosen for this work for various reasons. Taking cuttings is a simple operation compared to the other techniques. It is less labour intensive, less costly, and plants raised from cuttings establish themselves more easily than those raised by other methods. Where a stump produces many healthy shoots, many new plants can be raised from very few stockplants. Taking and treating cuttings for rooting does not require the skills necessary in grafting and budding. The low-technology non-mist propagators (Leakey *et al.*, 1990; Newton *et al.*, 1992a) used here, are much easier to construct than the modern laboratories necessary for micro propagation of cells and tissues in test tubes. Most of the species used in this study are more amenable to propagation by stem cuttings than they are to the other techniques. Grafting and air layering are also important techniques, particularly for promoting early fruiting but their principles of application are not well understood for these species.

The development of successful protocols for the vegetative propagation of these species by cuttings will enable a principal limitation to their domestication to be overcome, namely the low availability of planting material. Once techniques for multiplying clonal material have been developed, then these could possibly be combined with grafting techniques to produce clonal orchards with dwarf stature and early fruiting.

1.10 Factors affecting rooting of leafy stem cuttings

Success in propagation by leafy stem cuttings depends on adventitious root formation and development (Salisbury and Ross, 1978). Rooting is known to be influenced by a large number of interrelated factors (not yet defined) (Leakey *et al.*, 1989b; Mesén, 1993; Wells, 1955). Some of these factors have been identified to include the state and age of the stockplant, clonal variation, the stockplant growth environment, cutting origin, (post-severance) treatments applied to cuttings, propagation environment and season (Leakey *et al.*, 1994). According to Andersen (1986), these factors may be divided into pre- and post-severance factors.

Great differences exist between plants of different species in the rooting ability of cuttings taken from them. Leafy stem cuttings of some species root so readily that the simplest facilities and care give high rooting percentages, while other species can be rooted only if optimum conditions are maintained during rooting (Hartmann and Kester, 1983). Some of the factors which are crucial in rooting leafy stem cuttings of tropical hardwoods are discussed below.

The rooting process of cuttings

A cutting removed from a stockplant, under appropriate conditions, has the ability to produce a new root system and finally become an entire plant (Lovell and White, 1986). When leafy stem cuttings of woody and non-woody plants are taken, the cells at the basal cut surface die and suberin is formed to seal the wound and plug the xylem from infection and desiccation (Hartmann and Kester, 1983). The living cells behind the suberin plate differentiate to produce a mass of parenchyma cells called callus (Lovell and White, 1986; Hartmann and Kester, 1983). In most species, phloem, xylem and cambium parenchyma cells dedifferentiate to produce a meristematic condition (Salisbury and Ross, 1978; Lovell and White, 1986), which then form root primordia (Hartmann and Kester, 1983). It is from these root primordia that adventitious root initials are formed, and tracheids and sieve tubes are developed to link up with those in the xylem and the phloem in the cutting stem. In other species where the root primordia already exist, the primordium cells may give rise to the initials of organized root tips (Stangler, 1956). Subsequently, vascular connections are formed between these root initials and the existing vascular tissue of the cutting stem before functional roots emerge (Lovell and White, 1986).

Priestley and Swingle (1929) confirmed the view that in herbaceous plants, adventitious roots usually originate between the vascular bundles. For example, in tomato, pumpkin (Petri, Mazzi and Strigoli, 1960) and mung bean (Blazich and Heuser, 1979), adventitious roots arise in the phloem parenchyma, while in *Crassula* they arise in the endodermis (McVeigh, 1938) and in *Coleus*, they originate from the pericycle (Carlson, 1929). In woody perennial plants where one or more layers of secondary xylem and phloem are present, adventitious roots in stem cuttings usually originate from living parenchyma cells, primarily in the young, secondary phloem, but sometimes from such other tissues as vascular rays, cambium, phloem, lenticels or pith (Ginzburg, 1967; Mahlstede and Watson, 1952). In some plant species, adventitious roots form in the callus at the base of stem cuttings. This phenomenon has been observed in pine (Severova, 1949), common yew, Canadian hemlock yew and Podocarpus where the active tissues between the wound cambium and the cork phellogen take part in the development of root primordia (Komissarov, 1969). In this instance, a connective system is developed between the callus and the stem of the cuttings. In plant propagation, this type of rooting, with the formation of root primordia in the callus, is rarely encountered and is mainly found in species that root with difficulty, and particularly in conifers (Komissarov, 1969). It is, therefore, not surprising that in most kinds of plants, callus formation and the formation of roots are independent of each other (Hartmann and Kester, 1983).

During root emergence, roots from deep primordia break the ring of pericycle fibres and then the external tissues of the bark as well. In some cuttings, roots emerge through the callus, possibly because of the lower mechanical resistance of this tissues (Komissarov, 1969). Root emergence occurs at various points on the basal end of the cuttings. In cuttings of several species, such as large-leaved hydrangea, Japanese spindle tree, fuzzy deutzia, several species of *Viburnum*, roots appear not only near the cut surface, but also on the entire internode (Komissarov, 1969). The emergence of roots between callus and bark is observed in cuttings of Japanese aucuba, Balearic box, common box, willowlike callistemon and other broadleaved, as well as coniferous species. However, root emergence has been found to be obstructed by certain anatomic structures such as sclerenchyma bands, secretory canals, resin canals, or large volumes of induced vascular tissue (Lovell and White, 1986).

The role of carbohydrates on rooting of cuttings

Carbohydrates are the principal source of energy for protein and nucleic acid synthesis and other biosynthesis (Haissig, 1974, 1982), and the main substrate for production of carbon skeletons during rooting. In most cuttings, carbohydrates are present in greater concentrations than alternative energy sources such as lipids (Haissig, 1974). Eliasson (1978) doubled the root number in pea cuttings by supplying a 1% sucrose solution to the rooting medium, and Loach and Whalley (1978) increased rooting by 33% by supplying a 2% sucrose solution to cuttings of various woody species. Carbohydrates have been known to play additional roles in adventitious root formation such as influencing osmoregulation, cellular solvent capacity and other physiochemical phenomena (Haissig, 1986). This indicates that endogenous carbohydrate content may be a limiting factor during the rooting process of cuttings.

It is, therefore, not surprising that the carbohydrate content of cuttings has long been considered an important determinant of their rooting success (Okoro and Grace, 1976). Substantial evidence suggests that cuttings root best under conditions that yield optimum internal total non-structural carbohydrate concentrations before and after rooting (Reuveni and Adato, 1974; Andersen *et al.*, 1975; Reuveni and Raviv, 1981; Haissig, 1982; Leakey, 1983). However, the optimum carbohydrate content in cuttings for optimum rooting have not been defined under any conditions (Haissig, 1986; Veierskov, 1988).

The influence of carbohydrate concentration on the rooting process has been the source of some controversy (Davis and Potter, 1981), even for the most studied species such as pea (Veierskov and Andersen, 1982; Veierskov, Hansen and Andersen 1976; Veierskov *et al.*, 1982). During the rooting process of cuttings, carbohydrate concentrations may be influenced by endogenous and applied auxin which can enhance the mobilization of carbohydrate in leaves and the upper stem, or increase transport to the rooting zone (Nanda, Kochhar and Gupta, 1972; Altman and Wareing, 1975; Patrick and Wareing, 1973, 1976; Middleton, Jarvis and Booth, 1980; Veierskkov and Andersen, 1982; It has also been found that carbohydrate is translocated from the leaves and accumulated in the buds and the upper parts of the cutting stems before and during rooting (Haissig, 1982), and also accumulates in the developing roots (Okoro and Grace, 1976). The importance of a high carbohydrate content at the site of rooting has been shown in many cuttings, and generally wherever morphogenetic processes take place (Stoltz and Hess, 1966; Thorpe and Murashige, 1970). For example, carbohydrate accumulation at the cutting base occurred while the cuttings of ten different clones of avocado (*Persea americana*) were rooted under mist, and was correlated with their rooting capabilities. This view was confirmed by Haissig (1984) who reported that total carbohydrate accumulation in upper and basal stems of jack pine seedling cuttings stimulated root formation at basal ends during propagation.

It has been assumed that photosynthesis by cuttings positively influences rooting (Davis, 1988), although there is little scientific evidence to support or reject this assumption. Some authors have suggested that photosynthesis by cuttings during propagation is not an absolute requirement for root formation because leafy stem cuttings from a number of species can form at least some roots when placed in the dark (van Overbeek *et al.*, 1946; Eliasson and Brunes, 1980; Davis and Potter, 1981; Haissig, 1986). This is further supported by the rooting of etiolated tissue cultures (Fabijan *et al.*, 1981) and the rooting of non-photosynthetic leafless hardwood cuttings of many plants (Davis, 1988; Fabijan *et al.*, 1981). However, dark-propagated cuttings may not always root (Haissig, 1986).

Recent evidence suggests that the availability of current photosynthates may be important for rooting of some species of leafy cuttings (Haissig, 1986). This accords precisely with the results obtained by Newton *et al* (1992b) who found that the presence of current assimilates was important in the root development of *Terminalia spinosa*. There are many other examples to support this view. Davis and Potter (1981) found that rooting of leafy pea cuttings was reduced by about 50% if photosynthesis decreased to compensation point by manipulating light intensity, carbon dioxide concentration, or by applying an anti-transpirant. Reuveni and Raviv (1981) also found that the continuation of photosynthesis under mist led to accumulation of carbohydrate at the base of the cuttings, and this enhanced the rooting of avocado (*Persea americana*). In a more recent study on cutting stem respiration during rooting of *Prosopis juliflora*, Dick *et al* (1992) suggested that root development could be completely inhibited by insufficient sugars at the base of the cutting.

In pea cuttings, Eriksen (1973) observed that current photosynthates started to accumulate only after root initiation several days after excision of the cuttings from the stockplant. This is in accordance with the results of Okoro and Grace (1976), who, after their work on *Populus tremula*, stated that root production in cuttings influences photosynthesis and not the reverse, although the results of Leakey and Coutts (1989) on *Triplochiton scleroxylon* are contrary to this view. A model has recently been developed by Dick and Dewar (1992) based on the results derived from rooting the cuttings of this species. This model describes the role of carbohydrates in three different pools, which illustrate the flow of sugars at all stages to the rooting zone where they are needed for active metabolism at the cutting base. Tissue respiration rates have been reported to be higher here than any other part of the cutting thereby creating a concentration gradient which tends to pull sugars basipetally from source of production, mainly in the leaves (Dick *et al.*, 1992).

1. PRE-SEVERANCE FACTORS AFFECTING ROOTING OF LEAFY STEM CUTTINGS. STOCKPLANT GROWTH ENVIRONMENT.

The effects of stockplant growth environment on the successful rooting of cuttings has received increased attention in the last eight years (Moe and Andersen, 1988). The physiological condition of the stockplant is the result of the interaction between genotype and environmental factors such as light, temperature, water, carbon dioxide and mineral nutrition (Moe, 1977).

i) Effects of temperature on stockplants and subsequent rooting of cuttings

The rate of many plant metabolic processes is controlled by temperature, and therefore the temperature of the stockplant growth environment may have a direct effect on rooting. However, relatively few investigations have attempted to determine the optimal temperatures for rooting. Heide (1964) found out that in *Begonia*, temperatures within the range 12 to 22°C had relatively little influence on the mass of roots produced on single leaf cuttings. Leafy stem cuttings of *Campanula isophylla* had a higher dry mass if taken from stockplants grown at higher (18 to 22°C) temperatures (Heide, 1964), but there was no difference in the rooting of cuttings. Using a model system consisting of young pea plants, Kaminek (1962) found that stockplants grown at very low temperatures (3-4°C) yielded cuttings that formed more roots than those grown at higher ambient temperatures. Fischer (1981) carried out a series of temperature experiments with a leafy pea cutting system and found that there was a delay in development of four to five days if the temperature of the stockplants was decreased from 25 to 15°C. Andersen

(1979) stated that an abrupt change in temperature at the time of taking the cutting affects subsequent metabolism. Factorial experiments conducted by Fischer (1981) showed no effect of moving cuttings from stockplants grown at one temperature to a rooting medium at another temperature on root formation or on the length of the rooting period.

The available evidence, based on experiments with very few species, points to temperature during stockplant plant growth having only minor effects on rooting of cuttings (Andersen, 1986; Moe and Andersen, 1988). Low temperatures, however, promote rooting in some species. This generalization seems to apply to stockplants grown within a range of about 12-27°C (Moe and Andersen, 1988). These views are shared by Wells (1955) who stated that, in a greenhouse situation, cuttings taken from stockplants which had been chilled (in the case of temperate plants) rooted much more readily than if not chilled.

ii) Influence of irradiance on stockplants on subsequent rooting of cuttings. Intensive controlled experiments have been conducted on the effect of irradiance during stockplant growth on the rooting of several species (Heins *et al.*, 1980; Moe and Andersen, 1988). There is strong evidence from these studies, including dahlia (Biran, 1973), pea (Hansen *and*, 1974; Hansen, 1976) and hibiscus (Johnson and Hamilton, 1977a) among many other species, that a relatively low light intensity (irradiance) of the stockplant gives higher rooting percentages than is obtained with cuttings taken from stockplants grown at a high light intensity. However, Andersen (1986) stated that the effect on subsequent rooting after exposing the stockplants to different irradiances is variable, because increased irradiance may inhibit, delay, promote or have no effect on rooting. Stockplants require a certain minimal irradiance to give cuttings that root well, and the optimal irradiance for stockplants may vary from species to species and sometimes between cultivars (Moe and Andersen, 1988). Leakey and Storeton-West (1992) found that cuttings of *Triplochiton scleroxylon* rooted successfully (>60%) from stockplants grown at low irradiance of 106 μmol m^{-2} s^{-1}, but much less successfully at a higher irradiance of 246 μmol m^{-2} s^{-1}. Some plants fail to form roots at very low (almost darkness) irradiances (Andersen, 1986). The reasons for relatively high rooting percentages at lower irradiances is not well understood (Hartmann and Kester, 1983; Moe and Andersen, 1988). It has been suggested that at sub-optimal stockplant irradiances rooting may be limited by lack of carbohydrate and auxin supplies to the base of cuttings during rooting (Moe and Andersen, 1988; Nanda *et al.*, 1971; Hansen *et al.*, 1978). At supra-optimal irradiances, carbohydrate concentrations may be associated with photo-destruction of auxin, and possibly changes in water relations and concentrations of rooting inhibitors and/or promoters (Moe, 1977).

iii) Effect of stockplant light quality on rooting of cuttings

Very little attention has been paid to the influence of light quality on rooting, although it has been demonstrated by Heins and Wilkin (1979) and Moe (1977) that light sources with a high R:FR ratio stimulated lateral branching of stockplants and were favourable for cutting production in some herbaceous and woody plants such as roses. However, Leakey and Storeton-West (1992) reported that the subsequent rooting percentage of cuttings taken from stockplants of *T. scleroxylon* grown under R:FR ratios of 1.6 was higher than those from a R:FR ratio of 6.3. Hoad and Leakey (1992) also found that the rooting percentage was significantly higher (30-47%), and mortality lower, in cuttings from stockplants of *Eucalyptus grandis* raised at R:FR ratios of 0.4 and 0.7 than those raised at 3.5 and 6.5. Leakey and Storeton-West (1992) pointed out that the independent effects of light quality and irradiance on rooting ability provides some evidence that photosynthesis is an important determinant of rooting ability.

iv) Effect of stockplant mineral nutrition on subsequent rooting of cuttings

The mineral nutrition of stockplants can exert a strong influence on the development of roots and shoots from cuttings (Pearse, 1943; Preston, Shanks and Cornell, 1953; Samish and Spiegel, 1957). Stockplant nutrition also influences root, cutting and shoot quality (Pearse, 1943; Knoblauch, 1976; Hentig and Roeber, 1976; Reuther and Roeber, 1980; Roeber and Reuther, 1982). This influence depends on the physiological state of the stockplant tissue (Hartmann and Kester, 1983). For example, Kraus and Kraybill (1918) observed that yellowish stem tomato cuttings, high in carbohydrate but low in nitrogen content, produced many roots but shoots with low growth rates, while those with green stems, containing higher nitrogen contents, produced few roots and shoots with high percentage survival. Green stems, with very low carbohydrate but high nitrogen content, all decayed without producing either roots or shoots (Kraus and Kraybill, 1918). In large scale propagation work, it is necessary to apply fertilizers with the correct balance to stockplants. Hartmann and Kester (1983) suggested that to achieve this, nitrogen fertilizers to the stockplants should be reduced, thus reducing shoot growth and allowing for carbohydrate accumulation. However, studies conducted with grapes (Pearse, 1943, 1946) showed that cuttings obtained from stockplants grown under P, K, Mg or Ca, deficiency rooted less well than those from stockplants receiving complete nutrient applications; but with a reduced nitrogen supply to the stockplants, rooting increased.

Since there are limited data on mobilization studies showing movement of particular mineral nutrients, specifically N into the base of cutting (Stuart, 1938; Strydom and Hartmann, 1960) and P (Good and Tukey, 1966), root initiation may be influenced primarily by the initial nutrient contents within the base of a cutting. In this case, nutrient addition offers an appropriate method of correlating rooting data with particular stockplant nutrient availabilities. Blazich (1988) stated that although the results of fertilization treatments may show increased percent rooting and increased root number, which may be interpreted as reflecting greater root initiation, these data are but an indirect measure of root initiation which takes place only on a cellular level, except if this is linked up with anatomical or similar studies. Haun and Cornell (1951) and Preston *et al* (1953), examined the influence of stockplant (NPK) availabilities of rooting of geranium (*Pelargonium hortorum*) and azalea

(*Rhododendron*) respectively. The results showed that N had the greatest influence on root initiation, and the low and medium availabilities resulted in higher rooting percentages than the high availability in both experiments. On the whole, rooting response of cuttings to N was greater than to P and K, since varied levels of P and K had no influence on rooting.

v) Effect of shoots from different parts of the stockplant on rooting cuttings

The physiology, morphology and subsequent rooting potential of a cutting is influenced by the position of the shoot within the stockplant canopy (Leakey *et al.*, 1992b; Leakey, 1983, 1985; Tchoundjeu, 1989). For many forest species, cuttings from the lower crown root as well or more successfully than those from the upper portions (Black, 1973; Girouard, 1970; Grace, 1939). In *Triplochiton scleroxylon* stockplants of 0.5 m height, higher rooting percentages were associated with cuttings from the top shoot, and progressively declined with each shoot down the plant. In taller stockplants (1.5 m) treated with NPK, rooting percentages of cuttings from upper shoots were low and those from basal shaded shoots were relatively high (Leakey, 1983). The importance of shading was confirmed by the results of an experiment conducted on *Triplochiton scleroxylon* by Leakey and Storeton-West (1992), (see earlier). In another West African Tropical hardwood species, *Lovoa trichilioides*, rooting percentages of cuttings from basal shoots were highest, even in relatively short stockplants (Tchoundjeu, 1989). This variation in rooting of cuttings from different shoots has been attributed to various causes such as competition among shoots, shading of basal shoots by apical ones, nutrient gradients from apical to basal parts of the shoots, changes from juvenile to mature tissue from the apical to the basal end of stockplants and accompanying increases in the degree of lignification in the same direction (Leakey, 1983; Leakey *et al.*, 1992).

vi) Effect of different cutting positions within the shoot on rooting

Leakey (1983) found that the percentage rooting of cuttings of *Triplochiton scleroxylon* decreased sequentially from 70% for apical cuttings to 10% for the basal nodes. Leakey and Mohammed (1985) also reported that cutting length and rooting ability of the same species decreased basipetally down the length of the shoot.

This variability within the same shoot may be due to the fact that water, soluble carbohydrate, starch, nutrients and growth regulator contents may decrease down the shoot (Leakey *et al.*, 1992). However, basal cuttings with larger diameters rooted highest, suggesting that cutting volume, and thus perhaps storage capacity for assimilates, may in fact be the critical characteristic (Leakey and Mohammed, 1985; Leakey *et al.*, 1992).

Rooting of leafy stem cuttings is generally influenced by node position (Leakey, 1985). In *Triplochiton scleroxylon*, the rooting ability of single-node leafy cuttings decreased markedly down the shoots. This could be due to any of the many differences between cuttings of different positions on the shoot (Leakey, 1983). Cutting leaf age on the same shoot increases from the tip to the base of the shoot. Dostál (1956) stated that growing leaves and those that have just ended their growth stimulate root formation in cuttings, whereas old leaves hinder it. Experiments in rooting leafy stem cuttings of *T. scleroxylon* indicated that carbohydrate contents and water potential gradient in the leaf down the shoot is reversed in cuttings after two weeks' propagation (Leakey and Coutts, 1989). The amount of light reaching the leaves is greater the higher the shoot. Lignification and secondary thickening increase from the top to the bottom of the shoot. Nitrogen content and probably growth regulators such as auxin, cytokinin and gibberellins may also vary from node to node down the same shoot. These factors affect the rooting of cuttings from different portions of the same shoot.

vii) Effect of stockplant water relations on subsequent rooting of cuttings

Watering stockplants too much or too little may respectively lead to waterlogging or drought both of which are detrimental to the subsequent rooting of cuttings (Andersen, 1986). Circumstantial evidence indicates that cuttings taken from stockplants in a wet season root more successfully than those taken in a dry season (Cameron and Rook, 1974). The suggestion that cuttings fully turgid at the outset root more rapidly is likely to be true, but has not been rigorously tested (Loach, 1988). Recent investigations with *Milicia excelsa (Chlorosphora)* showed that cuttings with high relative water content did not all root even after they were given the same irrigation treatment (Ofori, 1994). This indicates that turgid cuttings do not necessarily root well. It does not, however, imply that cuttings from droughted stockplants will root. Studies with both cacao (Evans, 1952) and pea (Rajagopal and Andersen, 1980) cuttings showed reduced rooting when the cuttings were taken from stockplants displaying a water deficit. Consequently, plant propagators recommend that cuttings be taken early in the morning or in cloudy weather when the plant material is in a turgid condition (Hartmann and Kester, 1983).

viii) Effect of juvenile and mature plant material during rooting

The ontogenetic age of the stockplant can be an overriding factor in root formation. Either stem or root cuttings taken from plants in the juvenile growth phase, as found in young seedlings, often form new roots much more readily than those taken from plants in the adult growth phase, whether seedlings or vegetatively propagated plants (Gardner, 1929; Sax, 1962). Experiments with apple, pear, eucalyptus, oak, Douglas fir, and many other species have shown that the ability of cuttings to form adventitious roots decreased with increasing age of the plant from seed (Hartmann and Kester, 1983). In an investigation of the rooting response of cuttings of *Acacia mangium* from stockplants of four different ages (6, 12, 18 and 24 months), Ahmad and Pirrie (1990) found that percentage rooting decreased markedly with age after the first year (57, 52, 25 and 12% respectively). The results of a more recent study showed that the rooting percentage of the cuttings of 1, 2, 10 and 20 year-old stockplants of *Milicia excelsa* declined significantly with age (68, 13, 1 and 0% respectively) (Ofori, 1994).

Any treatment that maintains the juvenile growth phase is of value in preventing the decline in rooting potential as the stockplant ages (Hartmann and Kester, 1983). In rooting cuttings of difficult species it is useful to be able to induce rejuvenation of plants in the adult form. There has been a general consensus that the upper parts of a mature tree form the reproductive organs and lower, chronologically older parts retain the juvenile characters, referred to as the juvenile zone (Wiltshire and Reid, 1992; Borchert, 1976). In recognition of this concept, hedging and pruning have been applied to stockplants in an effort to obtain juvenile shoots for propagation work (Bonga, 1981; Leakey *et al.*, 1982b).

Recent studies have shown that in woody and non-woody plants, ontogenetic, physiological and chronological ages have different effects on rooting, irrespective of zonal location (Borchert, 1976; Hackett *et al.*, 1992; Wiltshire and Reid, 1992; Leakey *et al.*, 1992). Differing chronological ages of shoots on any part of the same plant indicate that the term "juvenile phase" is of doubtful value (Borchert, 1976). Thus many of the reported effects of phase change on rootability, particularly those comparing shoots collected from different parts of the same tree, may be attributable to physiological ageing rather than to epigenetic effects of sexual maturation (Leakey *et al.*, 1992).

2. POST-SEVERANCE FACTORS INFLUENCING ROOTING

i) Effect of auxin application on rooting of cuttings

The treatment of cuttings with substances which promote adventitious rooting dates to the earliest (1934-35) attempts to vegetatively propagate plants (Blazich, 1988). The relatively specific root-promoting properties of applied natural and synthetic auxins indicates that these substances play a crucial role in the rooting process (Gaspar and Hofinger, 1986). In particular, the process of root initiation in cuttings is auxin-sensitive (Maynard and Bassuk, 1986). In 1935, for example, it was first reported that the synthetic auxins IBA and IAA had strong-root promoting properties

(Zimmerman and Wilcoxon 1935). Auxins are now commonly used as an aid in rooting cuttings in all areas where plant propagation work is carried out. In some cases, auxins are known to increase the percentage of cuttings that form roots, hasten root initiation, increase the number and quality of roots produced per cutting and to increase the uniformity of rooting (Hitchcock and Zimmerman, 1936). However, cuttings from different species respond differently to different auxin concentrations. It has been shown by Hamzah (1992) that the cuttings of *Hopea odorata* gave 70% rooting without any auxin treatment, and it has been reported that auxin treatment had no effect on rooting percentage of *Lovoa trichilioides* (R R B Leakey, personal communication). Although Leakey *et al.* (1982b) reported that the optimum dose is generally around 40 μg per cutting, different doses of IBA have been found to be optimum for the cuttings of different tropical hardwood species

ii) Effect of leaf area on rooting

There is considerable experimental evidence (Rappaport, 1940; Went, 1929) indicating that the presence of leaves on cuttings exerts a strong stimulatory influence on root initiation (Cooper, 1935), and that root development is connected with leaf activity (Komissarov, 1969). Experiments have further shown that leaf retention on cuttings enhances rooting, and there is a positive correlation between rooting percentage and leaf retention (Reuveni and Raviv, 198). For example, leafless cuttings of *Terminalia spinosa* did not root, but cuttings with leaves gave rooting percentages of up to 81% (Newton *et al.*, 1992b). It has generally been assumed that photosynthesis during rooting provides carbohydrates to the base of the cutting (Davis, 1988). During the rooting process, the leaves play additional roles on the cuttings such as production of auxin (Breen and Muraoka, 1973) and control of water status (Komissarov, 1969; Leakey and Coutts, 1989).

Water losses are minimized by stomatal closure, but closure limits photosynthesis by reducing carbon dioxide intake. Droughting of cuttings may be minimized by providing a humid environment for rooting and by reducing the cutting leaf area

(Leakey and Coutts, 1989). However, optimum leaf area for rooting varies with species. For example, an optimal leaf area of 50 cm^2 was found in *T. scleroxylon* (Leakey, 1982b) and of 10-30 cm^2 in *K. ivorensis* (Asanga, 1989; Tchoundjeu, 1989). In some species, there is no tendency towards an optimal leaf area such as in *Terminalia spinosa* (Newton *et al.*, 1992b) and *Nauclea diderrichii* (Leakey, 1990).

3. THE ROOTING ENVIRONMENT OF CUTTINGS

After taking the cuttings, they can be inserted in one of a variety of propagation systems (Hartmann and Kester, 1983). High variability in rooting, or in some cases complete failure to root may occur if adequate environmental conditions are not maintained during propagation (Loach, 1988). In this study, a non-mist propagation system was used (see Newton and Jones, 1993a; Leakey et al., 1990; Mesén, Newton and Leakey, 1993).

i) Effect of humidity and vapour pressure deficit on rooting cuttings

Successful rooting of cuttings depends on maintaining high leaf water potentials (Loach, 1977). Water loss from the leaf is directly proportional to the water pressure deficit between the leaf and the surrounding air (Gay and Loach, 1977). Higher turgor of the cutting leaf can be achieved by increasing the humidity of the propagation environment. It is suggested that the humidity inside the propagator polythene should be near saturation, such as when there is condensation on the internal surfaces (Loach, 1977). To maintain high humidity inside a non-mist propagator, the propagator must be watertight and adequately shaded. The lids should be closed most of the time, since vapour pressure deficit increases with opening of the propagator lid in the non-mist system, (Newton and Jones, 1993a).

ii) Effect of irradiance during propagation on rooting

The irradiance in the rooting environment of leafy stem cuttings can significantly influence rooting ability (Andersen, 1986; Loach, 1988; Grange and Loach, 1983). Hartmann and Kester (1983), for example, pointed out that in all types of plant growth and development, light is of major importance as the source of energy in photosynthesis, and that in rooting cuttings, the products of photosynthesis are important for root initiation and growth. However, high irradiance may reduce the concentration of water vapour in the air surrounding the leaves of cuttings. This reduction may lead to an increase in vapour pressure deficit in the rooting environment, resulting in increases in transpiration, which could affect rooting adversely. High leaf irradiance may raise the cutting leaf temperature, thereby accelerating water loss and water deficits in the cuttings. Water deficits may reduce rates of photosynthesis, and therefore, production of photosynthates. Consequently, low light intensity has been advocated for rooting (Loach and Gay, 1979). Experimental evidence that cuttings of some plants root successfully under relatively low irradiance was shown many years ago (Stoutemyer and Close, 1946) in studies with inflorescent lamps. The results were confirmed in more recent studies with blueberry (Waxman, 1965), forsythia, viburnum, and hibiscus cuttings (Loach, 1979). Very high irradiance may damage leaves on the cuttings, delay rooting, and reduce root growth (Hartmann and Kester, 1983). The concentration of rooting inhibitors may be higher under high irradiances, although this effect has not been proven (Hartmann and Kester, 1983).

iii) Effects of temperature on rooting

Many experimental results show that temperature is one of the most decisive factors influencing adventitious root formation in cuttings (Pravdin, 1938). For example, the cuttings of *Theobroma cacao* rooted best at an average temperature of 25°C (within a range of 20-30°), while all cuttings died when the temperature fluctuated between 8 and 20°C. Petyaev (1955) stated that the optimum temperature for rooting cuttings of olive trees varies from 16 to 20°C, and a slight rise in temperature reduces rooting sharply. Andersen (1986) observed that the optimal temperatures for rooting were considerably higher for those cuttings which came from stockplants grown under high temperatures. It has also been shown that optimum temperatures for the formation of callus and roots vary in cuttings of the same plant. For example, the optimum temperature for the formation of callus in cuttings from 3-10 year-old branches of apple (Springdale variety) proved to be 26-28°C, while for the formation of roots it was 34-38°C (Swingle, 1929). Some studies have revealed that the optimum temperature for rooting cuttings varies with their degree of lignification. Unlignified cuttings of myrtle, red escallonia, rosemary and grape myrtle, for example, rooted more successfully at a lower (20-22°C) than at a higher temperature (27-30°C), whereas a higher rooting percentage (96-100%) was obtained from semi-lignified cuttings of the same species at the higher temperature of 27-30°C (Swingle, 1929).

The relative temperature of the air and rooting medium may also influence rooting. *Triplochiton scleroxylon* (Leakey *et al.*, 1982b) cuttings rooted more successfully with a higher substrate than air temperature. Hartmann and Kester (1983) stated that in cutting beds, thermostatically controlled heat applied below the cuttings is beneficial in maintaining the temperature at the base of the cutting higher than that of the buds, which promotes rooting rather than shoot development. Cuttings of most plants root more successfully at relatively low air temperatures (15-27°C) (Andersen, 1986; Brown, 1951; Fischer, 1981; Eriksen, 1973), although some root better still at temperatures much lower than these (Hartmann and Kester, 1983). There is a conflict of opinion about the optimum temperature for rooting. Loach (1988) stated that suboptimal temperatures in the rooting media tend to favour bud development occurring in advance of root formation. Similarly, Hartmann and Kester (1983) indicated that excessively high air temperatures tend to promote bud development in advance of root development, and to increase water loss from the leaves, and may therefore be detrimental to rooting.

iv) Effect of mineral nutrition on rooting of cuttings

After separation from the stock plant, cuttings have a fixed mineral nutrient pool, except for any minerals absorbed from the rooting medium or irrigation solution (Haissig, 1986). During root initiation in cuttings, stimulated by auxins or other co-factors, any nutrient involved in the metabolic processes associated with dedifferentiation and root meristem formation is essential for root initiation. Such a complex process probably requires most nutrients used required by plants for growth in general (Blazich, 1988; Eliasson, 1978; Haun and Cornell, 1951). Mengel and Kirby (1982) stated that mobilization of a mineral nutrient into the base of a cutting during root initiation is a strong indication that the nutrient is important in this stage of the rooting process. However other studies have not provided a clear picture of the importance of specific nutrients essential to root initiation (Blazich, 1988). Stuart (1938) and Strydom and Hartmann (1960), for example, carried out mobilization studies on mineral nutrients within cuttings during rooting of kidney bean (*Phaseolus vulgaris*) and plum (*Prunus avium*) respectively and found that N was mobilized during root initiation in the cutting stems, and its redistribution was accelerated by auxin treatment. In contrast Blazich and Wright (1979) and Blazich *et al.*, (1983) detected neither N mobilization nor any redistribution of P, K, Ca and Mg during root initiation in stem cuttings of Japanese holly (*Ilex crenata*). However, Good and Tukey (1967) reported mobilization of P but not N, K, and Ca during root initiation in cuttings of chrysanthemum (*Chrysanthemum morifolium*). Differences in reports about mobilization of particular nutrient elements during root initiation may be related to species differences (Blazich, 1988). It has been shown that Mn was negatively correlated with rooting capability in avocado (*Persea americana*) cuttings, while N, P, K, Ca, Mg, Na, Cl, B, Fe and Zn were positively correlated with the rooting capability of this species (Reuveni and Raviv, 1981). In addition to its positive correlation with rooting capability in cuttings of avocado, boron has been shown to be essential for rooting of mung bean cuttings (Middleton *et al.*, 1978).

After initiation, cutting roots still require mineral nutrients for growth and development. Movement of mineral nutrients to the bases of the cuttings following root initiation is an indication that these nutrients are needed for root growth and development (Blazich, 1988), although one study showed that a critical number of roots must be initiated before mobilization occurs (Blazich *et al.*, 1983). These authors also observed that certain nutrients such as Ca and Mg are sometimes mobilized from the base to support growth in the upper cutting stem.

It is very likely that mineral nutrient of leafy stem cuttings are, during rooting, particularly in a mist propagation system where the sprayed mist may leach the leaves (Evans, 1951; Sharpe, 1955). Studies have shown that N, P, K, Ca and Mg are leached from cuttings while under mist (Good and Tukey, 1966; Blazich *et al.*, 1983), with greater leaching occurring in hardwood cuttings than in softwood or herbaceous cuttings (Good and Tukey, 1966). Greater leaching from hardwood cuttings has been attributed to an increased proportion of the nutrients being in an exchangeable form, whereas in young, growing tissues nutrients are quickly metabolized within cells which are difficult to leach (Good and Tukey, 1966). Other factors such as mobility of a particular nutrient may also influence this phenomenon (Tukey *et al.*, 1958).

Loss of mineral nutrients through leaching during mist propagation has the use of nutrient-enriched mist to replenish the lost nutrients. Results have been mixed. Wott and Tukey (1967) reported that rooted cuttings of various herbaceous, softwood and hardwood species under nutrient-enriched mist resulted in increased root quality and greater top growth than propagation under water-only mist. However, nutrient mist propagation had a deleterious effect on cuttings of several azalea cultivars, resulting in injured foliage and inhibition of rooting (Keever and Tukey, 1979). Nutrient mist also caused sanitation problems by causing the growth of algae (Wott and Tukey, 1967; Coorts and Sorensen, 1968).

Controlled release fertilizers either top-dressed or incorporated into the rooting medium have been used during mist propagation, as an alternative to nutrient mist. Although this method enhanced overall root quality and subsequent cutting growth (Johnson and Hamilton, 1977; Ward and Whitcomb, 1979), percent rooting was unaffected (Johnson and Hamilton, 1977). These results may also be related to the inability of unrooted cuttings to absorb nutrients. It was observed that in chrysanthemum (*Chrysanthemum morifolium*) only small quantities of P were absorbed from the medium even though P availabilities were high (Wott and Tukey, 1969), in contrast to the absorption of large quantities by the foliage applied through nutrient mist. The influence of nutrient leaching on rooting, post-propagation growth and vigour has not been clearly established (Blazich, 1988). Excessive leaching resulting from the growth stage of the cutting (Good and Tukey, 1966) or from other factors (Tukey *et al.*, 1958), may be critical.

v) Effect of rooting media during rooting of cuttings

In vegetative propagation practice, rooting media are considered to be an integral part of the propagation system (Loach, 1988). An ideal rooting medium provides sufficient porosity to allow aeration, has a high water-holding capacity yet is well drained, is free from harmful pathogens (Hartmann and Kester, 1983), and has a pH of 6.5-7.5 since acidity tends to inhibit rooting (Bruckel and Johnson, 1969). Cuttings of many species root easily in a variety of rooting media but those more difficult to root may be greatly influenced by the kind of rooting medium used, not only in the percentage of cuttings rooted, but also in the quality of the root system formed (Long, 1932). Materials such as sand, peat, sphagnum, vermiculite, perlite and soil have each been used as a rooting medium, and sometimes used in combinations (Hartmann and Kester, 1983).

vi) Effects of water availability on rooting of cuttings

A central feature of the behaviour of leafy cuttings is that lacking roots, they readily develop water deficits (Loach, 1988). It has long been recognized that turgor must be maintained in cuttings to achieve rooting (Loach, 1988). Measurements have shown that low water potentials (well below -1.0 MPa) often occur in cuttings, and such low values are related to low rooting percentages (Loach, 1977). Evans (1952) indicated that even a slight water deficit which may be insufficient to cause any visual symptoms of distress, results in a considerable delay or a reduction in rooting. Free amino acids and sugars may accumulate during droughting of the cutting (Barnett and Naylor, 1966; Stewart, 1971). Stomatal closure, which may be induced by water deficits and abscisic acid, affects carbohydrate gain by photosynthesis directly by reducing diffusion of carbon dioxide to the chloroplasts, and indirectly by causing a rise in leaf temperature (Loach, 1988). In addition, water deficits in cuttings affect cell growth and synthesis of new components, auxin concentrations in the cutting tissue (Boyer and Wu, 1978), cell wall synthesis and cell division (Hisao, 1973), translocation of photosynthates to developing root primordia (Loach, 1988), and may diminish the supply of leaf co-factors known to synergize the action of auxin in promoting adventitious root formation at the base of the cutting (Hess, 1969).

Although cuttings of different species vary in their water requirements, maintenance or gain in water content by cuttings may generally be a prime determinant of successful rooting (Evans, 1952; Loach, 1988; Gay and Loach, 1977; Newton and Jones, 1993a). Salisbury and Ross (1978) point out that there is an optimum selative water content (RWC) above and below which plant functions are arrested. Water loss from cuttings can be reduced by s

content of the rooting media, the use of anti-blocking chemicals to reduce stem resistance to water uptake and by wounding the cuttings of species which are more prone to developing water deficits (Grange and Loach, 1983; Loach, 1988). The maintenance of high humidity around the cuttings is of vital importance in maintaining turgor (Newton and Jones, 1993a).

CHAPTER 2

GENERAL MATERIALS AND METHODS

CHAPTER 2

2. 0 Materials And Methods

2.1 Study Area

This study was carried out in Kumba in Meme Division in the South West Province of Cameroon. Meme Division lies approximately 400 m above mean sea level. The Division covers a surface area of about 6190 km^2, and lies between 4°12' to 5°29' N and 9° to 9°55' E. Villages near Kumba were selected for a preliminary socio-economic survey which led to the fruit and vegetable studies of the experimental species. The Southern Bakundu forest reserve where the main experiments were carried out lies approximately 234 m above mean sea level, between 4°22' to 4°34' N and 9°16' to 9°27' E. The reserve is situated at a point fifteen kilometres to the west of the major road from Kumba to Buea and covers an area of 194.25 km^2 (Figure 2.1 A, B, C and D). An all-season motorable forest road radiates from this point westwards through the Kendongi Forestry Research Camp to the research plots from where it narrows down to a forest path which leads to Ekombe. Here it joins the main road to Mundemba where the Korup Project is currently being carried out (See Korup Project details later). On both sides of this road between the camp and the research plots, locations were selected for the collection of *Gnetum africanum* vines, and selection of trees of *Ricinodendron heudelotii* and of *Irvingia gabonensis*. This selection was directed by the local vegetable and fruit collectors who come from nearby villages and the families of the research and forest service workers.

The climate of this area is characterized by two main seasons. The rainy season extends from April to September with October being the transition from wet to dry, and the dry season extends from November to February with March being the

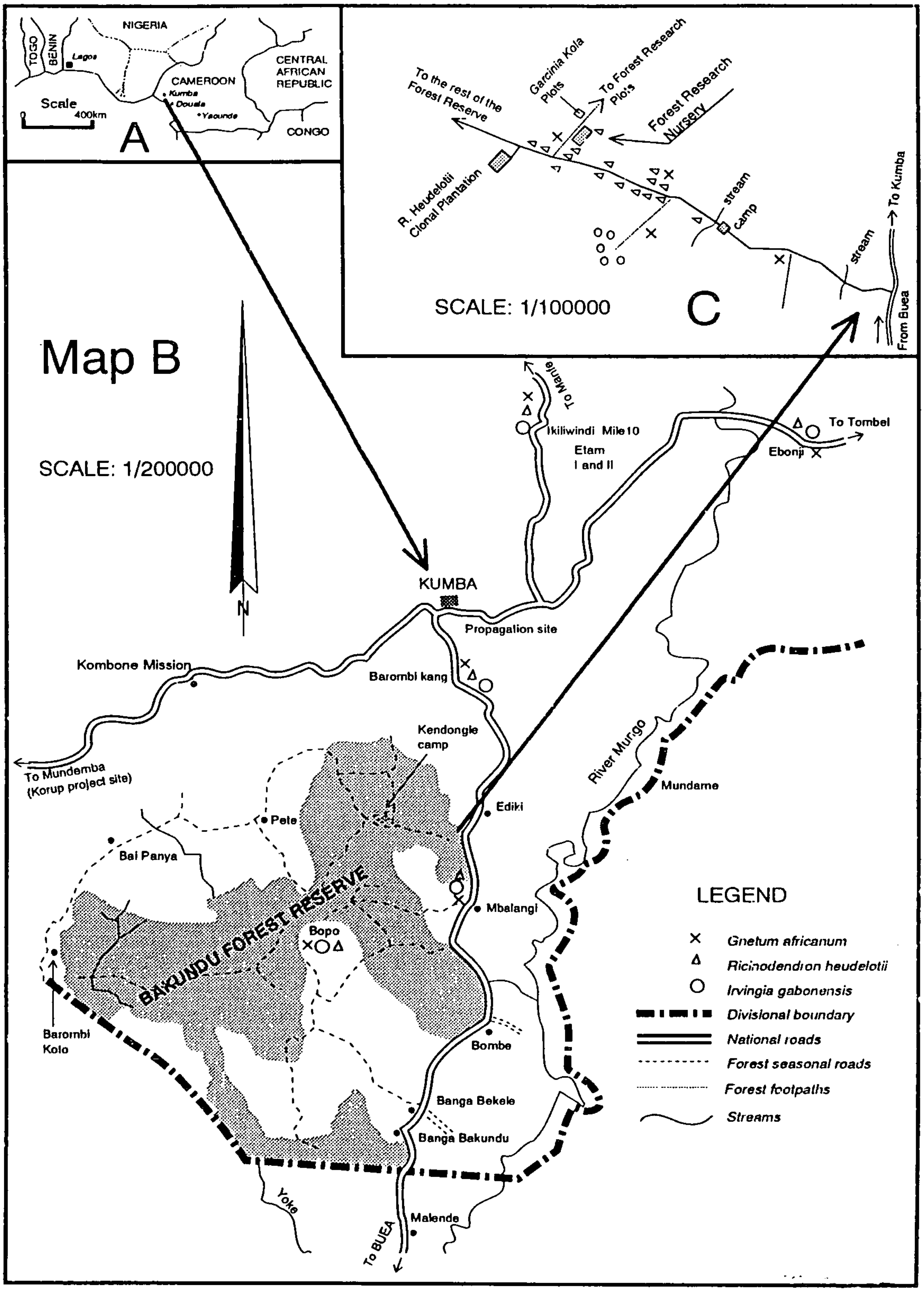


Figure 2.1 A, B, C and D

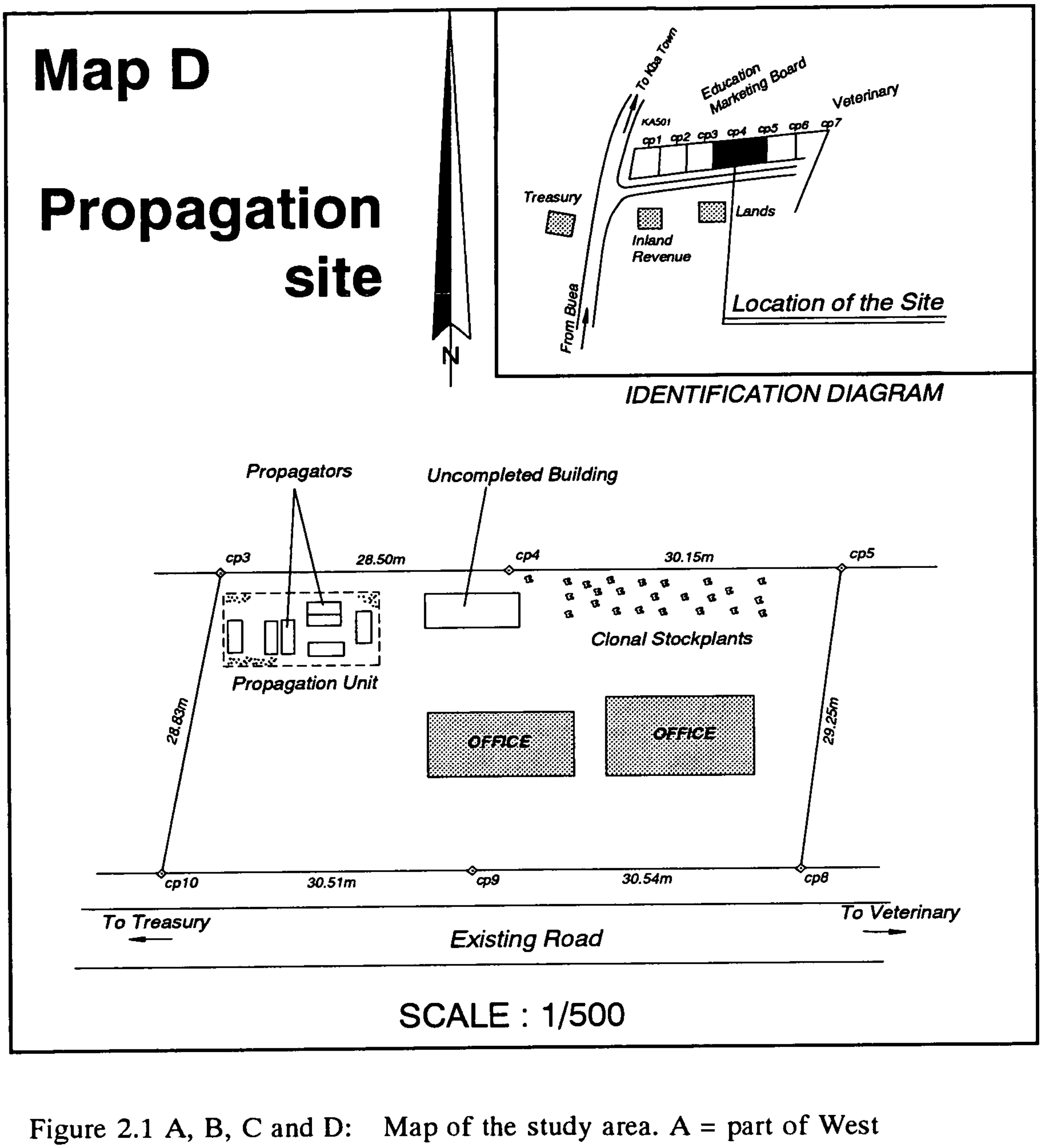


Figure 2.1 A, B, C and D: Map of the study area. A = part of West Africa, B = Southen Bakundu Forest Reserve C = study site in the Reserve and D = the propagation unit in the premises of the Station for Forestry Research, Kumba, Cameroon.

transition from dry to wet. In general, the study area experiences 4-5 months of dry season and 7-8 months of rainy season. The rainy season usually has a dry spell of a week or two in July or August during which maize is planted.

The climatic data presented here were taken from records of a local meteorological station installed at Kendongi camp in the forest reserve. The rainfall for 1992 was 2065 mm and distributed over all the months of the year (Figure 2.2 A). The temperature for the same year ranged from 20.65 °C to 33.3 °C with a monthly mean of 28.8 °C, and relative humidity ranged from 77.9% to 91.6% with a monthly mean of 83.9% (Fig. 2.2A & B). From records kept since 1977, rainfall of the reserve and surrounding areas ranges from 1800-2600 mm yr^{-1}. Mean monthly temperature falls between 25-30°C with lower the temperatures occurring in the rainy season. The mean relative humidity taken at 13.00 h is generally high throughout the wet season (exceeding 85%) and drops to 60% and below throughout the dry season. December, January and February are the driest and July, August and September the wettest months of the year, although there are some variations from year to year.

The geology and soils of this area, which is situated to the northeast of Mount Cameroon (locally called Mt. Fako), are highly influenced by its volcanic activity. The last volcanic eruption took place about eleven years ago. A large part of the reserve lies on the typical Mount Cameroon black basalt rock series, which consists of compact and very fine dark grained basalto-andesitic rock of the Tertiary period (Geze, 1943; Richards, 1963) associated with its previous eruptions. A very small part of the reserve (northern tip) falls on what is usually called the Barombi Kang series, which outcrops in Kumba and stretches to the south and southeast and consists of ash and lapilli, often considered younger (Quaternary period) than the typical Mount Cameroon black-basalt series. The rocks of the area yield clay loam and are dark reddish grey (Munsell colour-10R 3/1) and dusky red (10R 3/2) on the surface to dark reddish brown (5 YR 3/4) at depth (60-100 cm) (Songwe *et al.*, 1988). Basalt outcrops are frequent and deposits of sand and gravel are often found in stream beds (Lamb, 1960).

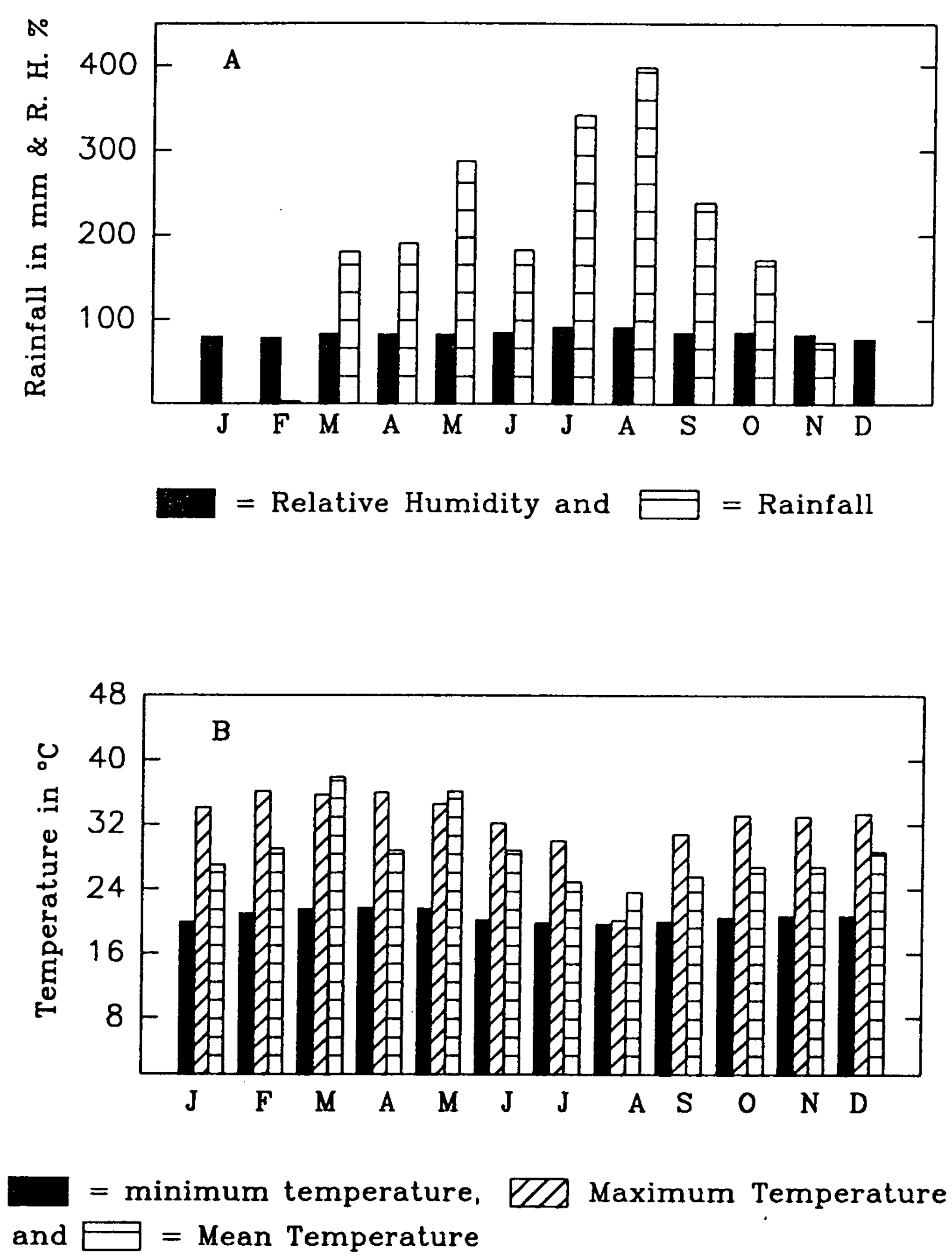


Figure 2.2 A & B: Graph of climatic data from study area (Southern Bakundu) for the year 1992.

Legal and illegal exploitation of the forests in the Division has resulted in heavy logging of desirable species in this reserve, so that what is now left is broken or fragmented forest. However, the forest that remains, although devoid of most species, can still adequately be described as tropical evergreen lowland forest of the UNESCO (1973) scale or moist semi-deciduous forest. A few upper and middle storey species make the forest appear evergreen, among which are *Desbordesia glaucescens, Staudtia stipitata* and *Pycnanthus angolensis,* and the species of these strata that are deciduous include, among others, *Terminalia superba, Celtis zenkeri, Ceiba pentandra, Piptadeniastrum africanum, Cylicodiscus gabonensis* and *Distemonanthus benthamianus.* The understorey species are many and diverse since very few of them are exploited for timber. Among these species are *Irvingia gabonensis, Garcinia kola* (both evergreen), *Ricinodendron heudelotii* (deciduous), *Xylopia aethiopica,* and *Uapaca* spp.(both evergreen). There are various kinds of climbers, some of which reach the crowns of the top storey trees and some which only reach crowns of the understorey trees, among which is *Gnetum africanum.* Among climbers of economic importance are rattans, *Gnetum* spp. and *Momordica angustisepala* (bush sponge). Principal among the ground flora are *Aframomum* spp. *Thaumatococcus daniellii,* and *Heisteria parvifolia.*

Korup is a high forest area situated on the Nigerian border in the south-west of Cameroon. It is in the Ndian division of the South West Province. It occupies an area of about 3200 sq. km. Within it is the Korup National Park which was created by a Presidential decree in 1986, as a protected area of 1260 sq.km. The Korup Project covers the whole of this area. This block of forest has not been exploited since it is inaccessible and the soils are infertile (IUCN, 1991). Korup contains over 3000 species of plants and vertebrate animals, and is a centre of biological diversity in Africa.

The major aims of the Korup Project are: (a) to prohibit entry and hunting in the Korup forest; (b) to recruit both more plant and animal species into the park, which already has a unique biodiversity of rare plant and animal species; (c) to conserve and manage the forest on a sustainable basis without interference from the local people who have depended on the forest for a long time for their livelihood.

The Korup project has developed a number of strategies aimed at keeping away the people living within and around the project area from entering the forest. One such strategy is to resettle the villagers at the periphery of the forest, also known as a buffer zone. Resettlement requires a rural development programme. This kind of programme obliges the local community to develop a sustainable land use system. An effective land-use system involves a sustainable farming system such as agroforestry. The results of this project will be relevant to the development of sustainable agroforestry systems, incorporating native fruit trees for implementation in the buffer zone of the Korup National Park.

2.2.0 The propagation unit

The propagation unit was built in the immediate vicinity of the Forestry Research Station at Kumba. Here, the unit is close to a water source, and is accessible to both people and vehicles. The unit is set on slightly sloping land on sandy soil which provides good drainage and ample working space, without mud. The area is spacious enough to contain the propagators and sufficient room to stand potted cuttings for nursing in readiness for follow-up operations. At the start of the study, three propagators with sloping roofs were constructed. Later on three additional propagators with flat tops were built to meet increasing research needs. A total of six propagators have, therefore, been built to date. The largest one is made up of six compartments of one square meter each, all under the same roof for media experiments. All six propagators were constructed following the same low-technology non-mist design described by Leakey and Longman (1988); Newton *et al.* (1992a), Mesén *et al.* (1993). This design consists essentially of a polythene enclosure made with a wooden frame, placed on a well drained bed of gravel, sand or sawdust (Plate

2.1).

The lids were made in little compartments of one square metre each in order to allow work to be done in one section of the same propagator without opening the whole thereby altering the relative humidity therein. This lid compartmentalization is an important improvement on non-mist propagator construction because opening the propagator causes a rapid decline in humidity, and this has an adverse effect on the cuttings set in the rooting medium (Leakey, 1989a; Newton *et al.*, 1992a).

2.2.1 Preparation of rooting media

Two main kinds of rooting media were used. They were rotted sawdust and clean sand obtained from the Mungo river (the river that forms the boundary between Anglophone and Francophone parts of Cameroon). In order to get particles of uniform size, and to remove unwanted wood pieces, the sawdust was sieved. Other rooting media were produced using different grades of sand. Details of the various media used in this study are given below. To protect them from fungal pathogens, a systemic fungicide (Benlate) was dissolved in water and sprayed on the surface of the media immediately after construction, and subsequent spraying was done if and whenever the need arose.

Fine and coarse river-washed sand was obtained locally. Sawdust rotted for over a six month period (the species was not known) was collected from a sawmill on the outskirts of Kumba. Sand was sieved through meshes of 2, 3 and 5 mm into three grades to constitute three rooting media. The sand that passed through the 5 mm mesh, but could not pass through the 3 mm mesh, was referred to as gravel; the sand that passed through the 3 mm mesh, but could not pass through the 2 mm mesh was regarded as medium sand, and the sand that passed through both the 3 mm and

Plate 2.1: Low-technology propagators in use and under constructionfor rooting multipurpose plant species in Kumba, Cameroon.

the 2 mm meshes, was referred to as fine sand. Sawdust was sieved through the 5 mm mesh. Equal quantities of gravel and sawdust were sieved through a 5 mm mesh to produce the gravel:sawdust (50:50) medium; this was repeated with medium sand to obtain the medium sand:sawdust (50:50) medium. The three grades of sand, sawdust and two mixtures gave six different media, which were placed in the propagator with six compartments for the media experiments (see Chapter Three).

2.2.2 Fencing and shading

The space occupied by the propagation unit was enclosed by a fence to keep out animals, both domestic and wild, and people. Wooden poles, 10 cm wide x 3 cm thick x 2.8 m long were erected around the unit in the form of a rectangle. Wire mesh was nailed on the poles, leaving a doorway, to protect the unit from any possible damage.

The tops of the erected poles were woven with wire to form a network on which one layer of shadecloth was placed and fastened at the edges with wire pegs. This shadecloth protected the unit from direct sunlight. The roof was later replaced by thick transparent plastic sheets in the centre and corrugated iron sheets at the edges. The shadecloth was rolled up and tied round the structure under the eaves, for unfolding at sun rise to protect the plants from direct lateral sunlight.

2.2.3 Rooting environment

Preliminary measurements of propagator microclimate were taken during November 1991, using a data logger and sensors. Detailed light measurements were later made between May 15th and August 7th 1993 with light sensors (Quantum Sensors, Sky Instruments Ltd., Llandrinddod Wells, Wales). Ten measurements were made in each of the six propagators, when a sensor was placed inside and also above the polythene covers of each propagator compartment. Ten readings were taken on ten different days, from the months of May, June, July and August, 1993, when the light sensors were available. Each time readings were taken in the propagators and within the propagation unit, a reading was taken in the open under the sky as a control. Irradiance data are presented in Table 2.1. These results indicate that approximately 25-55% of the light incident on the propagation unit is received on the surface of the propagators, and approximately 13-28% is received inside the propagators on the surface of the rooting medium (Table 2.1). The variation between propagators was quite pronounced, with propagator 4 receiving 13% of the control, whilst propagators 1 and 6, which were positioned nearer the edge of the unit, tended to receive higher percentages of 28% and 21% of the control irradiance respectively.

Temperatures of the rooting media inside the propagators were assessed during a 28 hour period from 5th to 6th November, 1991. Platinum resistant thermometer probes connected to a scientific data logger, model Cr 10 (Didcot Instrument Company Ltd., Campbell, USA) was inserted in each medium from 12.00 - 12.30 pm on November 5th, 1991 to 3.30-4.00 pm on November 6th, 1991, for 28 hours in total. The logger was programmed to take readings every ten minutes. The temperature data presented in Table 2.2 are the night and day mean readings, and the differences between the temperatures inside and outside the propagators are expressed in °C. The data presented in Table 2.2 are, therefore, those of November 1991.

Table 2.1. The amount of light received in the propagation unit and in the propagators in Kumba, during the period from May 15th to August 7th 1993. Values presented are maxima, minima and mean of ten measurements in μmol m^{-2} s^{-1} for each of the six propagators. The figures are means of the 3 compartments of each propagator, and of the 6 compartments in the case of the double propagator. Propagator 2 is double and contains six different rooting media. The 'control' value was measured outside the propagation area in an unshaded position (range of 3213-102 and mean of 2105 ± 331 μmol m^{-2} s^{-1} ($P < 0.05$; t-test)

	Measurements on propagator lid				Measurements on surface of rooting medium			
Propagators	Maximum	Minimum	Mean	% of control	Maximum	Minimum	Mean	% of control
1	1590 ± 72	219 ± 43	1165 ± 153	55	797 ± 23	141 ± 15	593 ± 24	28.
2	1511 ± 91	238 ± 31	1008 ± 140	47	811 ± 96	142 ± 10	484 ± 47	23
3	1279 ± 286	248 ± 45	700 ± 99	33	501 ± 18	125 ± 5	325 ± 7	16
4	896 ± 74	207 ± 45	534 ± 66	25	417 ± 97	120 ± 3	271 ± 29	13
5	1546 ± 80	178 ± 52	684 ± 160	32	841 ± 127	112 ± 12	363 ± 85	17
6	1147 ± 85	230 ± 18	833 ± 93	39	687 ± 62	133 ± 17	449 ± 33	21

Higher temperatures were maintained within the propagators both during the night and during the day than outside. The difference between the ambient temperatures and those in the media inside the propagators was higher during the night than during the day. However, night-time temperatures varied according to the medium in use, with fine sand displaying the lowest mean temperature difference compared to controls of 2.3°C, while sawdust displayed the highest value of 5.4°C. There was also a significant temperature difference between night-time and day-time means, and a pronounced variation in day means from one media to the other, with medium sand plus sawdust displaying the lowest mean difference of 0.1°C, while sawdust displayed the highest value of 3.4°C. The mean night-time temperature in the six media was 3°C higher than the control, whereas the mean day-time temperature was 1°C higher (Table 2.2).

Table 2.2 Temperature measured in six rooting media inside a propagator and outside under shade (control). These values are night-time and day-time means and differences between internal propagator temperatures and controls during a 28 hour period in November, 1991.

Media	ADTM	WPDTM	Mean difference (°C)	ANTM	WPNTM	Mean difference (°C)	WPDTM WPNTM	ADTM-ANTM
MS	23 ± 0.5	24 ± 0.2	0.6	20 ± 0.1	22 ± 0.1	2.4	1.3	3.1
MS:SD	23 ± 0.5	23 ± 0.2	0.1	20 ± 0.1	23 ± 0.1	2.6	2.5	3.1
FS	23 ± 0.5	23 ± 0.3	0.5	20 ± 0.1	22 ± 0.1	2.3	1.3	3.1
SD 77	23 ± 0.5	26 ± 0.3	3.4	20 ± 0.1	25 ± 0.1	5.4	1.1	3.1
SS:SD	23 ± 0.5	23 ± 0.3	0.4	20 ± 0.1	24 ± 0.1	2.5	1.0	3.1
Grand Means	23 ± 0.5	24 ± 1.3	1.0	20 ± 0.1	23 ± 1.3	3.0	1.0	3.1

(ADTM = ambient day-time temperature means (control), WPDTM = within propagator day-time temperature means, ANTM = ambient night temperature means, WPNTM within propagator night-time temperature means, G:SD = gravel plus sawdust, MS = medium sand, MS:SD = medium sand plus sawdust, FS = fine sand SD = sawdust).

The air humidity in the propagators was not measured. However, the propagators were constructed to minimise water loss. The water table was maintained at a depth of a few centimetres below the base of the cuttings using a hollow observation tube. In the mornings, it was observed that there was often condensation on the inner surface of polythene covers, indicating that the air was saturated overnight.

The air:water ratio of the different rooting media was measured by sampling approximately 100 cm^3 of each medium. Five samples of each medium were taken. The samples were each placed in a graduated plastic beaker to ensure that the volume was about 100 cm^3. Each was then carefully transferred to an aluminium dish, weighed, and the fresh mass recorded (Plate 2.2). Pure tap water was added from a pipette to the medium until saturated. The volume of water that displaced the air in the medium was recorded. For purposes of percentage determination of each medium component (air, water and solid), the dishes containing the medium and water were each weighed and the weight (total mass) of each dish was recorded. Each of the thirty dishes was placed in an oven (set at 95oC), one after the other, to dry for 48 h, then reweighed for estimation of dry mass. The air:water ratio was calculated as the ratio of the volume of air in the medium (estimated by the volume of water added to saturation) to the volume of water in the medium (fresh mass - dry mass).

The volumetric content of air in the medium was then calculated by multiplying the weight of the volume of air displaced by 100/total mass, that of solid by multiplying the weight of dry mass/total mass x 100 and that of water originally in the medium/total mass x 100 gave that of the water content of the medium. The results are presented in Table 2.3.

Plate 2.2: Volumetric water, air and solid content and air:water ratio determination of the rooting media used in the media experiments.

Table 2.3: Different media composition of air, water, solid and air:water ratio and their standard errors (n = 5).

Media	% Air	%Water	% Solid	Air:Water ratio
Sawdust	11.62	41.44	46.94	0.28 ± 0.02
Gravel:sawdust	7.20	36.02	56.78	0.20 ± 0.02
Medium sand:sawdust	5.01	21.04	73.95	0.24 ± 0.03
Fine sand	4.79	17.81	77.40	0.27 ± 0.03
Medium sand	6.34	37.63	56.03	0.17 ± 0.01
Gravel	5.31	17.86	76.83	0.30 ± 0.04

The calculations show that the air:water ratios of the media range from 0.17 in medium sand to 0.30 in gravel.

2.3 Plant materials

All the experiments carried out utilized cuttings taken from seedlings, vines and/or coppiced stumps. Eleven stumps of ***Ricinodendron heudelotii*** were coppiced in mid-March, 1991. Ten were located in Southern Bakundu forest reserve and one near the propagation unit at Kumba. Three trees from the Reserve were selected and designated clone numbers 2006, 2017 and 2034 while the tree grown near the propagation unit was given clone number 2001. The four trees successfully sprouted, and a number of cuttings were obtained from them and used mainly in the media experiment, and for bulking up clonal stockplants behind the propagation unit. Seedlings were obtained from natural regeneration underneath a mother tree near the Kumba Urban area. 48 of these were used in the auxin experiment, and the others in the leaf area experiments described later. For later experiments, cuttings were obtained from sprout shoots of coppiced plants of fourteen clones numbered 2001, 2006, 2015, 2032, 2034, AL1, AL2, AL3, AL4, AL5, AL6, AL7, AL8 and AL9 planted near the propagation unit in July 1991. For the last phase of this study, cuttings were obtained from sixteen

Ricinodendron heudelotii plants pollarded at four different heights in Southern Bakundu Forest Reserve in the later part of February, 1993. In order to reach these heights and manage the sprout shoots, a wooden scaffold, ladder and platform were constructed around the cut surface of each pollard.

Ten trees of *Irvingia gabonensis* were coppiced during mid-March 1991 in Southern Bakundu Forest Reserve. None of the stumps sprouted initially, although by early 1992, 5 had produced more than thirteen new shoots each. Cuttings for a media experiment were collected from these stumps in the third week of March, 1992. During the fruiting season (June to July) of 1991, seeds were collected from 3 sources - (1) Bakundu, (2) Mbalange and (3) Barombi-Kang. In Mbalange and Barombi-Kang villages, seeds were collected from trees growing on farms, while the others were collected from superior trees in the Southern Bakundu Forest Reserve. A total of 120 seedlings had been established in the nursery near the propagation unit by early 1992. This constituted the seedling stockplant source of *Irvingia gabonensis* cuttings for the leaf area and hormone experiments presented in Chapter four.

Cuttings of *Garcinia kola* were taken directly from newly flushed shoots on 3 mature trees in compartments 690 and 730 of the research plots in Southern Bakundu. One cutting out of the 50 taken successfully rooted. Later on, a number of other shoots were collected

Plate 2.3: Cloned *Ricinodendron heudelotii* from early experiments planted out in the field in Southern Bakundu Forest Reserve.

from the same plots. These shoots provided 140 cuttings, none of which rooted. Most of the seeds collected from the same research plots also failed to germinate. On the 16th of March 1991, ten trees were felled in the plots for the production of sprout shoots. Although some of the stumps subsequently sprouted, the sprouts failed to develop, so no cuttings were obtained from them. The seeds of this species failed to germinate to provide seedlings from where to obtain juvenile cuttings. *Garcinia kola* was, therefore, replaced with *Gnetum africanum* in the experimental programme.

Gnetum leaves are collected from various locations in the Forest Reserve in Southern Bakundu by "eru" collectors as mentioned earlier. The leaves are generally harvested from vines with broad leaves. Four of the major harvesting areas were randomly selected from where cuttings were taken for the media experiment. These site were numbered and referred to as locations 1, 2, 3 and 4. For subsequent experiments with this species, cuttings were obtained from the nearest harvesting sites (locations 2 and 4 mainly), which were easily reached on foot to save time between taking and inserting cuttings into the rooting media since the lone research vehicle for the Research Station had a break down and was, therefore, not available for field work. These sites were located at the same time when field tours were undertaken to locate the fruit species with the collectors, who gather fruits and harvest the leaves of *Gnetum* as well.

When cuttings were treated and set in the propagators to root, they were sprayed with a systemic insecticide and fungicide in mild doses dissolved in water, as prescribed by the manufacturer, to protect the cuttings from any form of pathogen attack. During this time, care was taken to ensure that the water table did not drop by observing through the hollow tube. The propagator lids were firmly fitted and opened only when necessary, in order to maintain a high humidity. Excess light was avoided by the use of the shadecloth. Seedlings and rooted cuttings were potted in polythene pots measuring 20 cm long and 15 cm in diameter. These pots were filled with top soil from a newly cleared area in the reserve near the research plots. The potted seedlings and cuttings were watered, to field capacity, twice a day in the mornings and evenings. When they were fully established in the small pots, the seedlings and cuttings were transplanted into larger polythene pots measuring 45 cm long and 25 cm in diameter for future propagation work or planting out in the field.

The nursery operations carried out while the plants were in the nursery consisted of watering, weeding, spraying with fungicides and insecticides where necessary, and staking in the case of *Gnetum africanum* to provide support for the climbing vines. Spraying with insecticide was required frequently for *Ricinodenron heudelotii* which was susceptible to psyllid attack, which tended to curl the leaves. Fertilizers were rarely used. However, during prolonged periods of watering, some liquid fertilizer was applied carefully to each pot.

Potted cuttings were weaned either in empty propagators or under shade for about three weeks. Two polythene frames were constructed and also used for weaning rooted cuttings. Each weaning frame measured 1.20 m wide by 3 m long. When it was ascertained that the cuttings were well established in the pots, the frames were completely taken off. After weaning, potted cuttings maintained under shade for some time before planting out in the field, as "hardening up" (Longman, 1993). Plate 2,3 shows some cloned *Ricinodendron heudelotii* from early experiments planted out in the a plot in the field in Southern Bakundu Forest Reserve, Kumba, Cameroon for growth studies and demonstration purposes.

2.3.1 Taking and preparation of cuttings

Cuttings collected in the field were taken from vigorously growing shoots very early in the morning. In the case of *Irvingia gabonensis*, sprout shoots were collected and placed on a polythene sheet placed above ice blocks, covered and taken to the propagation unit. *Gnetum* vines were harvested, rolled and placed in the ice box for conveyance. Sprout shoots of *Ricinodendron* were carefully placed in polythene bags, sprayed with water and taken at once to the propagation unit. In taking cuttings from stumps, orthotropic shoots were selected from the same level at the periphery of the cut surface. At the propagation unit, cuttings were obtained from the shoots using secateurs. Cutting lengths varied between 4 to 9.5 cm depending on the species and experiment.

In all the experiments in this study, an appropriate amount of indole-3-butyric acid (IBA) powder was weighed and dissolved into industrial alcohol to obtain the required dose. For a dose of 40 μg, for example, 0.04 g was dissolved in 10 ml of alcohol. A micrometer syringe was used in applying the hormone to the cut base of the cuttings, providing a drop of 10 μl. After application, the alcohol was quickly evaporated by a stream of cool air from a standing fan. The cuttings were then inserted into small holes in the rooting medium made with a blunt peg, to a depth of approx. 2.5 cm at equal spacing in lines, according to node position. After insertion, cuttings were sprayed with water, insecticide and fungicide to keep the leaves moist and to protect the cutting from pest or disease attack. The propagators were kept closed whenever possible, and once opened, the cuttings were sprayed with water from a hand-held sprayer. Under normal circumstances, cuttings were sprayed at 7.30 and after 17.30 hours daily for the first two weeks after insertion.

CHAPTER 3

Effects of different media, IBA concentration and leaf areas on rooting leafy stem cuttings of *Ricinodendron heudelotii*

CHAPTER 3

Effects of different media, IBA concentration and leaf areas on rooting leafy stem cuttings of *Ricinodendron heudelotii*

3.1.0 Introduction

In this chapter, the effects of three factors on rooting of leafy stem cuttings of *R. heudelotii* were examined, namely rooting media, auxin concentration and leaf area. These experiments were designed to define the treatments that would give optimum rooting results for eventual use in mass multiplication of this species.

3.2.0 Experiment 1: Effect of rooting media on rooting of leafy stem cuttings of *Ricinodendron heudelotii.*

3.2.1 Introduction

In rooting leafy stem cuttings, the rooting medium plays an important role in root initiation and development. Rooting media anchor the cuttings, enable them to take up water through the cutting base and are the substrate in which root initiation and development take place (Loach, 1985). After a comparative study of different rooting media, Loach (1988) observed that, although the nature of the medium can influence rooting to a great extent, as was also shown by Tchoundjeu (1989), rooting results differ between species. Differences between media may arise from the fact that some media, such as FS, tend to restrict the supply of oxygen to the cuttings base (Wells, 1955; Hartmann and Kester, 1983).

No study of the effect of rooting media on the rooting of leafy stem cuttings of *Ricinodendron heudelotii* has been carried out previously. The aim of the present experiment was to investigate the effect of six different media on rooting of leafy stem cuttings of this species from four clones. The media were sawdust, fine sand, medium sand, gravel, medium sand:sawdust and gravel:sawdust.

3.2.2 Materials and methods

In the propagation unit, propagator number two was divided into six compartments into which six different media were placed. Compartment (1) contained sawdust (SD) alone, (2) gravel (G) alone (3) 50:50 mixture of medium sand and sawdust (MS:SD), (4) 50:50 mixture of gravel and sawdust (G:SD), (5) medium sand (MS) alone and (6) fine sand (FS) alone. Time constraints and lack of plant materials made it difficult to replicate the six different media used in the media experiments in this study, in order to avoid treatment effect being confounded by changes in the rooting space in the propagator. Except where otherwise stated, these abbreviations will be used throughout this study for all media experiments. On February 12th, 1991, two lorry loads of fine and coarse river-washed sand were obtained locally as stated earlier. Equal quantities of G and SD were sieved through a 5 mm mesh to produce the G+SD (50:50) medium. This was repeated with MS to obtain the MS+SD (50:50) medium.

For the auxin application, 0.04 g of indole-3-butyric acid (IBA) powder was weighed on a sensitive balance, and dissolved in 10 ml of industrial alcohol. One drop of this solution drawn up into a micrometer syringe contained approximately 40 μg of the auxin, which was applied to the clean-cut cutting base. The cutting bases were then dried in a cool moving air stream from a fan for 1-3 minutes prior to insertion into the media.

By mid May, 1991, the stumps of clones 2006, 2017, 2037 and 2001 had produced coppice shoots, varying in number from twenty to thirty-five per stump. Three clones did not produce many shoots and five failed to sprout. These were excluded from this experiment. Ninety single-node cuttings, five from each shoot, were taken from each of the four clones. Leaves were trimmed to approximately 50 cm^2, using paper templates. Fifteen cuttings from each clone were inserted according to node position, into each of the six media, giving a total of 60 cuttings in each medium (5 cuttings x 3 shoots per clone x 4 clones). Cutting lengths varied between 6 and 9.5 cm. The cuttings were lightly sprayed with systemic insecticide and fungicide in accordance with the manufacturer's instructions. While the experiment was in progress, the cuttings were sprayed with water twice daily (at 7.30 and 17.30 hours) to keep the leaves moist.

Assessment

Assessments for callus root formation, leaf shedding, bud development, cutting mortality, root diameter and changes in cutting top and bottom diameters, were undertaken on a weekly basis starting from the second week after inserting the cuttings. Daily observations were made during all experiments for the incidence of dry leaf surfaces, cutting death, leaf shedding and propagator leakages.

Analysis

The six compartments containing the different media were placed in the same propagator. Analysis of variance was employed to assess differences between the six rooting media, followed by Fisher's t tests (LSD) at week five of the experiment using SAS (1980). Percentage rooting data were transformed by the formula arcsin $\sqrt{\%}$ prior to analysis. Standard errors and confidence limits of percentages were calculated following the procedure described by Snedecor and Cochran (1980) for binomial data.

Analysis of deviance by stepwise regression in GENSTAT 5 (Payne *et al.*, 1987) were applied to determine the effects of block, node position, stem length, stem diameter at the cutting bottom and treatment on the rooting ability of *R. heudelotii* cuttings at the last assessment.

3.2.3 Results

Percentage rooting

By the end of week one, no roots had developed in any of the six media. By the second week, cuttings commenced rooting in the media, with SD having a significantly higher (P <0.05; t-test) percentage of rooted cuttings than the other media. From week two onwards, percentage rooting increased between treatments, with SD maintaining the highest proportion of rooted cuttings throughout the experiment. Analysis of variance showed that there were significant differences (P <0.05) between the six media treatments in percentage rooting at the end of week five, although SD was not significantly different from FS at this same time (82% and 73% respectively) (Table 1A). Rooting percentage in FS was significantly higher than the other four media (with the exception of SD) in weeks three and four. Although rooting percentages in the other four media increased progressively from week two to four, there were no significant differences between them (P >0.05; t-test) (Figure 3.1a). When the data were analyzed by stepwise regression, rooting was found to be significantly affected by node position, but was not significantly affected by stem length and the six media treatments (Table A2). Clonal variation did not have a significant effect on the rooting ability of this species in this experiment.

Callus formation

The percentage of callused cuttings in all the six rooting media ranged from 62% to 90% by week two in G:SD and SD respectively, although no cutting in any of the other treatments formed callus after week one (Fig. 3.1b). Except for FS, SD and G were significantly higher (P <0.05; t-test) than the other media in terms of callus formation. However, the proportion of cuttings that callused did not differ significantly ($P > 0.05$; t-test) in FS, G:SD, MS and MS:SD. Generally, the percentage of callused cuttings increased with time from week two throughout the rest of the time the experiment was in progress (Figure 3.1b).

Cutting mortality

There was a marked difference in cutting mortality in the six media, with highest mortalities recorded in G:SD from week two to five. By the fifth week, percentage mortality in this medium was significantly ($P < 0.05$; t-test) higher than that in the other media except MS, in which 28% of the cuttings had died. Lowest mortality was recorded in SD, where less than 13% cuttings were dead by week five. However, there were no significant differences ($P > 0.05$; t-test) between SD and FS, although FS was significantly lower MS. Generally, percentage cutting mortality tended to increase with time from the first week throughout the five week observation period (Figure 3.1c). The main type of cutting mortality in this experiment was observed to be rotting.

Leaf abscission

Although leaf abscission was relatively high in the G:SD medium, it was not significantly different from that in the MS:SD mixture and MS in week one. The proportion of cuttings that lost their leaves was significantly lower ($P < 0.05$; t-test) in SD than in other media except G and FS, in which 12% leaf abscission each was recorded (Figure 3.1d). By week two, the cuttings in G:SD had lost 35% of their leaves. This loss increased to about 38% after four weeks. In FS, 15.1% of the cuttings lost of their leaves by week five (Fig.3.1d). Most of the cuttings that lost their leaves died. For example, by week five, the 40% of cuttings that had lost their leaves in the G:SD medium had all died.

Shoot formation

Except for the 2% cuttings in the MS medium which formed shoots in week one, cutting buds did not start developing into new shoots until week two, when 12% of the cuttings in FS and 2% in G had formed new shoots respectively. Percentage shoot formation rose very steeply between weeks two and three in all treatments. Shoot formation was significantly higher ($P < 0.05$; t-test) in SD than the other media, which were in turn not significantly different from each other except MS:SD, which was significantly lower than FS ($P < 0.05$; t-test) at week three. At the end of the five week assessment period, the highest percentage shoot formation (73%) was associated with SD, while the lowest (42%) was recorded in the MS:SD mixture (Figure 3.1e).

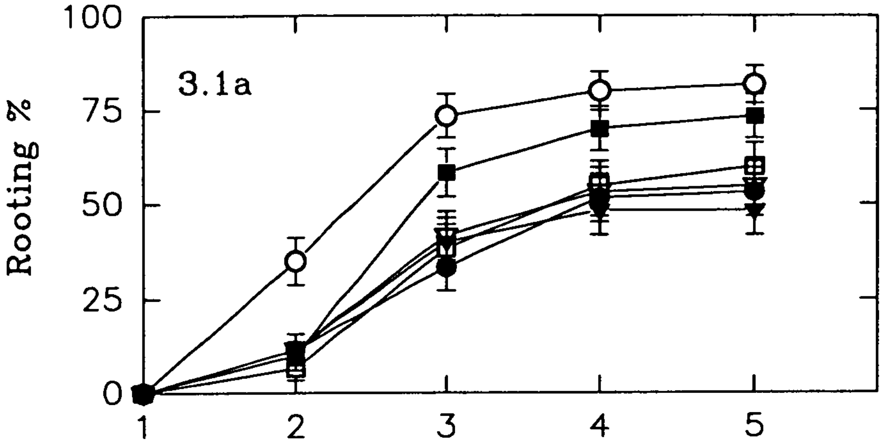
Number of roots per rooted cutting

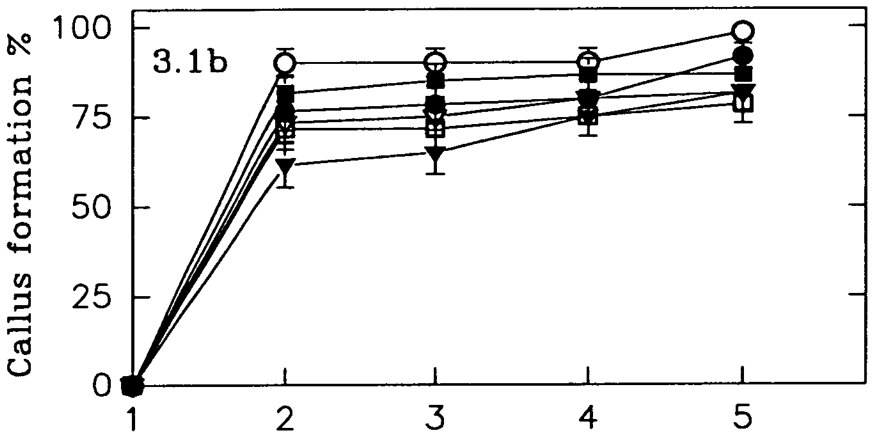
The mean root number per rooted cutting in SD did not differ significantly ($P > 0.05$; t-test) from that in FS. However, SD was significantly higher ($P < 0.05$; t-test) in this variable than the other four rooting media, in which there were no significant differences. After a five week rooting period, the highest mean (7.3) root number per cutting was associated with SD, while the lowest (3.8) was recorded in G (Figure 3.1f).

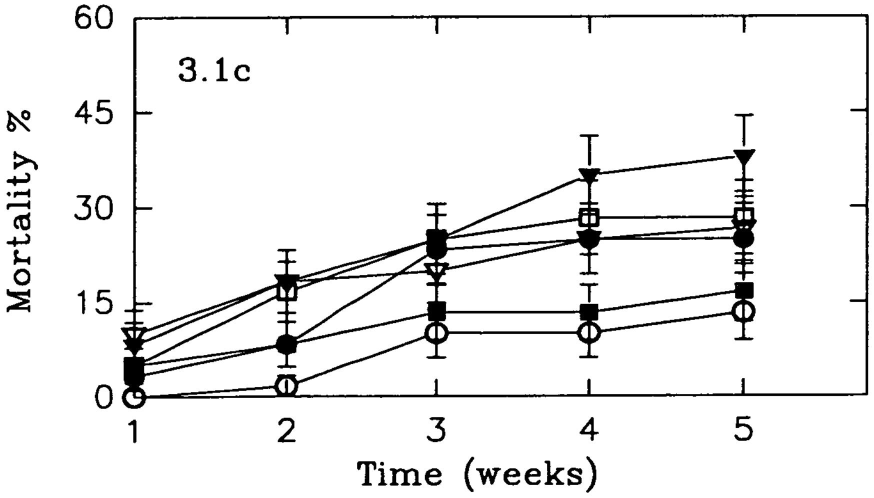
3.3. Experiment 2: Effects of different concentrations of indole-3-butyric acid (IBA) on rooting of leafy stem cuttings of *R. heudelotii*

3.3.1 Introduction

Maximum rooting of cuttings can occur if optimum concentrations of auxins are applied (Jarvis, Ali and Shaped, 1983; Shibaoka, 1971; Leakey *et al.*, 1982b). Such concentrations are often just below those which induce symptoms of toxicity


3.1a
Rooting %
100
75
50
25
0
1
2
3
4
5


3.1b
Callus formation %
100
75
50
25
0
1
2
3
4
5


3.1c
Mortality %
60
45
30
15
0
1
2
3
4
5
Time (weeks)

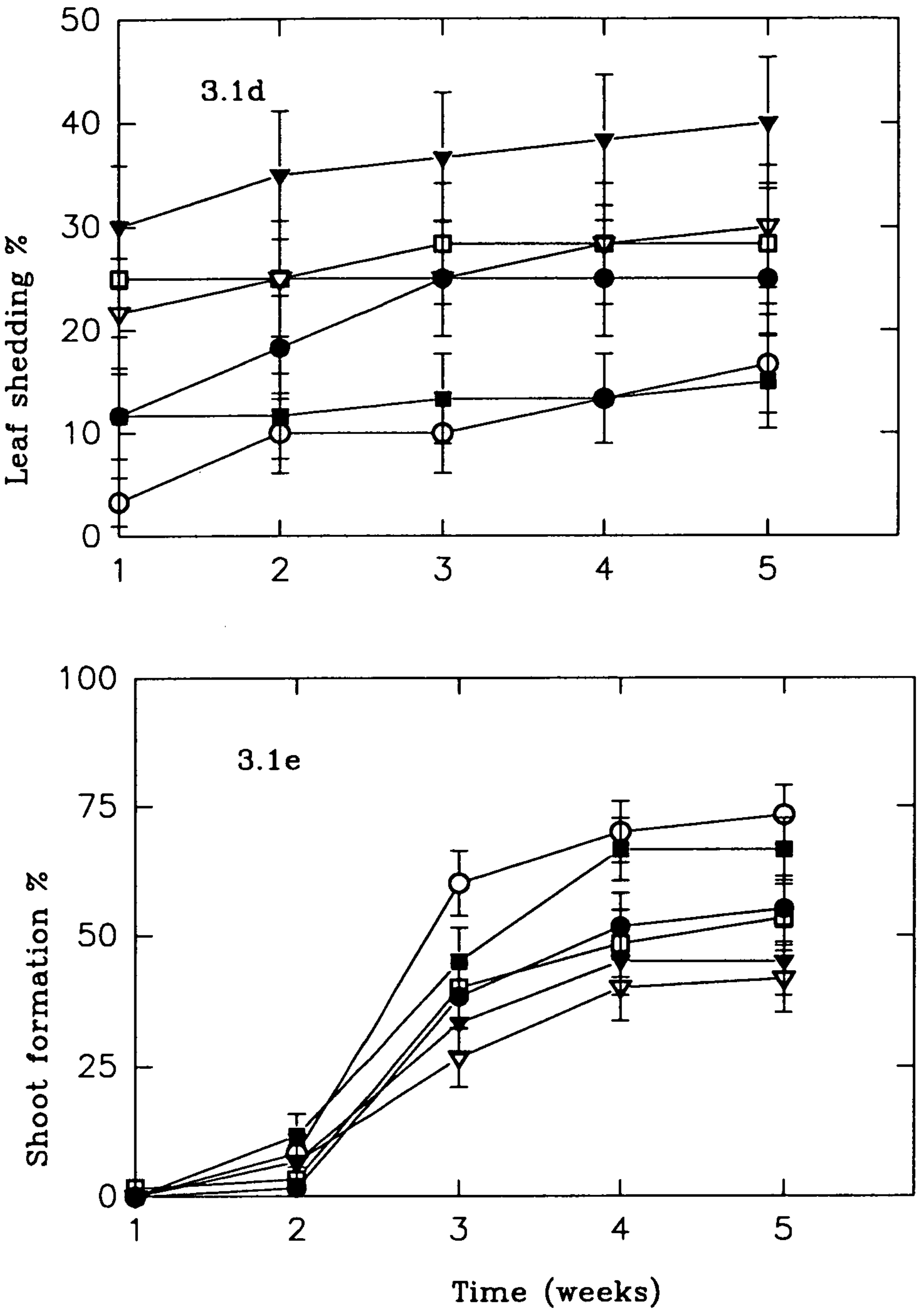


Figure 3.1: Effect of different media on rooting of leafy stem cuttings of *R. heudelotii* (○ = SD, ■ = FS, ▽ = MS:SD, □ = MS, ● = G and ▼ = G:SD) Bar = ± SE. (n = 60).

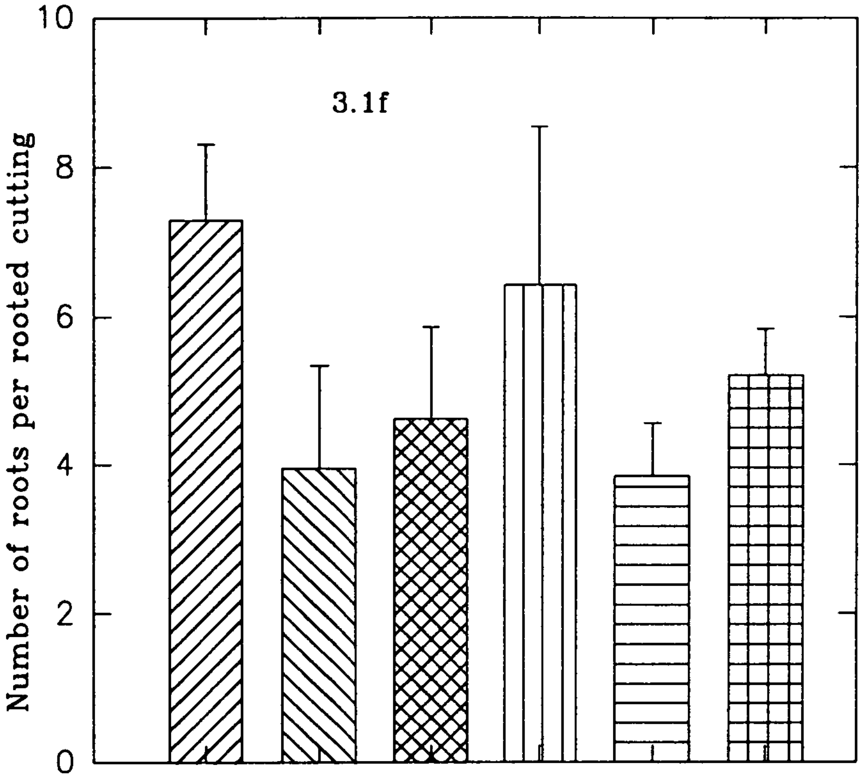


Figure 3.1f: Effect of media on number of roots per rooted cutting of *R. heudelotii*. (▨ = = SD, ▥ = FS, ▩ = MS + SD, ▤ = G and ▧ = G:SD) Bar = + SE. (n = 60). (5 weeks after insertion).

(Middleton, 1977; Jackson, 1970). For example, Leakey *et al.*, (1982b) showed that cuttings of some clones of *Triplochiton scleroxylon* had specific optima of 8 and 40 μg IBA per cutting respectively, while the rooting of some cuttings was found to be significantly inhibited by higher (200 μg) than optimum doses of IBA. Similarly, mortalities were found to be greatest in *Shorea macrophylla* cuttings treated with the highest (10800 *ppm*) concentration of auxin (IBA), which also results in a reduction in the number of roots per rooted cutting (Lo, 1985). The adverse effect of auxin concentrations above optimal values on leafy stem cuttings of *Dalbergia sericea* was also reported by Uniyal, Prasad and Nautyal (1993), who observed that of the 100 and 500 *ppm* (IBA and IAA) treated cuttings, those with a higher dose exhibited lower rooting percentage, decreased sprouting, reduced number of sprouts, roots and root length. However, Bhatt and Todaria (1993) reported that cuttings of *Debregeasia salicifolia* gave a rooting percentage of 50% when treated with 100 *ppm*, 0% when treated with twice this dose, but 60% when treated with 500 *ppm* of IBA.

Although some preliminary propagation trials with cuttings of *Ricinodendron heudelotii* were attempted in the U. K. (Leakey *et al.*, 1990), no effect of auxin on rooting cuttings of this species has been investigated to date. The objective of this experiment was to examine the effect of different IBA concentrations on rooting leafy stem cuttings of *R. heudelotii*, with the aim of selecting the optimum concentration for future mass production.

3.3.2 Materials and methods

Seedlings of *Ricinodendron heudelotii* were collected from an abandoned farm at about 12 km from the propagation unit south of Kumba on the 23rd of June 1991, then potted into small-sized (10 cm in diameter by 18 cm deep) polythene pots and nursed in the propagation unit covered with shadecloth (Tildenet LS 50%; Kerry PAR, Bristol, BS3 2HA, Kerrypack, UK). When the seedings had produced ten to fourteen leaves, cuttings were taken from each of them, and given one of four doses of IBA. The four doses were: 0 μg (control), 8 μg, 40 μg and 200 μg IBA, prepared by dissolving 0.008 g, 0.04 g and 0.2 g of IBA powder respectively, in 10 ml of industrial alcohol in each case. Four cuttings were taken from each one-shoot seedling stockplant. The four treatments were applied, randomly, to the four cuttings at the clean-cut stem base by the use of a microsyringe. Except where otherwise stated, each cutting was a single node with varying length. The leaves of each cutting were trimmed to approximately 50 cm^2. The four cuttings from each seedling stockplant were set randomly according to node position into blocks of sixteen cuttings of 4 treatments x 4 cuttings per shoot (4 node positions). This was repeated 10 times to constitute 10 blocks in the same propagator. There were 16 cuttings per block x 10 blocks giving a total of 40 cuttings per treatment. Assessments were made at weeks 2, 3, 4 and 5, after which rooting appeared to be complete.

Analyses

At the end of the four weeks of the experiment, analysis of variance was carried out to study the influence of auxin (IBA) concentration on the rooting percentage of the cuttings of *R. heudelotii*. This was followed by the LSD method of comparing differences between many means using Fisher's t test. The percentage rooting data was transformed by the formula arcsin √% prior to analysis. Standard errors of percentages were calculated following the procedure described by Snedecor and Cochran (1980) for binomial data.

Analysis of deviance for stepwise regression in Genstat 5 (Payne *et al.*, 1987) was utilized to determine the influence of auxin treatment, node position, cutting diameter and cutting length on the rooting ability of the cuttings of this species.

3.3.3 Results

Percentage rooting

Rooting did not start until the second week after insertion when percentages ranged from 30% in the 0 μg treatment, to 45% in the 8 μg auxin concentration. Percentage rooting increased gradually over the four week rooting period in all treatments. By week three, rooting percentage in the 8 μg treatment was significantly ($P < 0.05$; t-test) higher than in the other treatments, which were not significantly different from each other. After week four, rooting percentage was not significantly different in the 8 μg treatment ($P > 0.05$; ANOVA), with an overall percentage of 88% from the other treatments (Table A3). At higher concentrations of IBA, rooting percentage progressively decreased, such that the lowest percentage of 60% was recorded in the 200 μg treatment (Fig. 3.2a).

To investigate the relative influence of auxin treatment, block, stem diameter at the cutting base, node position and stem length on the rooting ability of cuttings, the percentage rooting results of cuttings were analyzed by stepwise regression. The analysis at the end of the experiment showed that rooting was significantly influenced by cutting diameter at the base and node position, while the influence of block and treatment on the rooting ability of the cuttings of this species was not significant (Table A4). Cuttings from the upper parts of the shoots, with thinner diameters were better rooters than those from lower stem.

Callus formation

By week two, there were significant differences between the treatments with respect to callus formation, with treatment 0 μg displaying a lower proportion of cuttings with callus than the other treatments. The highest percentage (63%) was associated with treatment 8 μg. This pattern persisted to week four (Fig.3.2b). Between week three and four, the percentage of callused cuttings remained unchanged in treatment 200 μg, while that of cuttings in the other treatments increased. At week four, percentage of callused cuttings was still significantly higher ($P < 0.05$; t-test) in the 8 μg treatment than it was in the others, between which there were no significant differences ($P > 0.05$; t-test).

Cutting mortality

One week after insertion, between 3% to 25% of cuttings had died per treatment, with the highest mortality associated with the highest IBA concentration of 200 μg and the lowest recorded in the 8 μg treatment. Cutting mortality did not exceed 5% and 8% in the 8 μg and control treatment respectively throughout the experiment. Mortality in the 200 μg treatment was significantly higher ($P < 0.05$; t-test) than in the other treatments from week two to four (Fig. 3.2c). Cutting mortality was significantly lower in the control treatment than in the other treatments, with the exception of the 8 μg treatment.

Leaf shedding

The highest proportion (32%) of cuttings which shed leaves at week one was recorded in the 200 μg treatment. In the second and third weeks, 15-52% of the cuttings treated with 8 and 200 μg shed their leaves, although there were no significant differences between these two treatments. By week four, 60% of the cuttings treated with 40 μg of IBA had lost their leaves. However, by week four, there were no significant ($P > 0.05$; t-test) differences between the four treatments with respect to leaf loss (Figure 3.2d). Early leaf shedding was associated with higher auxin doses while late leaf abscission was related to lower doses.

Shoot formation

Cuttings did not start to form new shoots until week two after insertion, when a definite trend was displayed, with percentage shoot formation increasing with increasing IBA concentration. There were no significant differences between the four treatments in week two, but by the third week shoot formation in the control treatment (0 μg) was significantly higher, and in the 200 μg treatment significantly lower, than the other two treatments. By week four, although the control and 8 μg treatments did not differ, they were found to be significantly higher ($P < 0.05$; t-test) than the other two treatments (40 and 200 μg IBA), which in turn did not differ significantly ($P > 0.05$; t-test) from each other (Figure 3.2e).

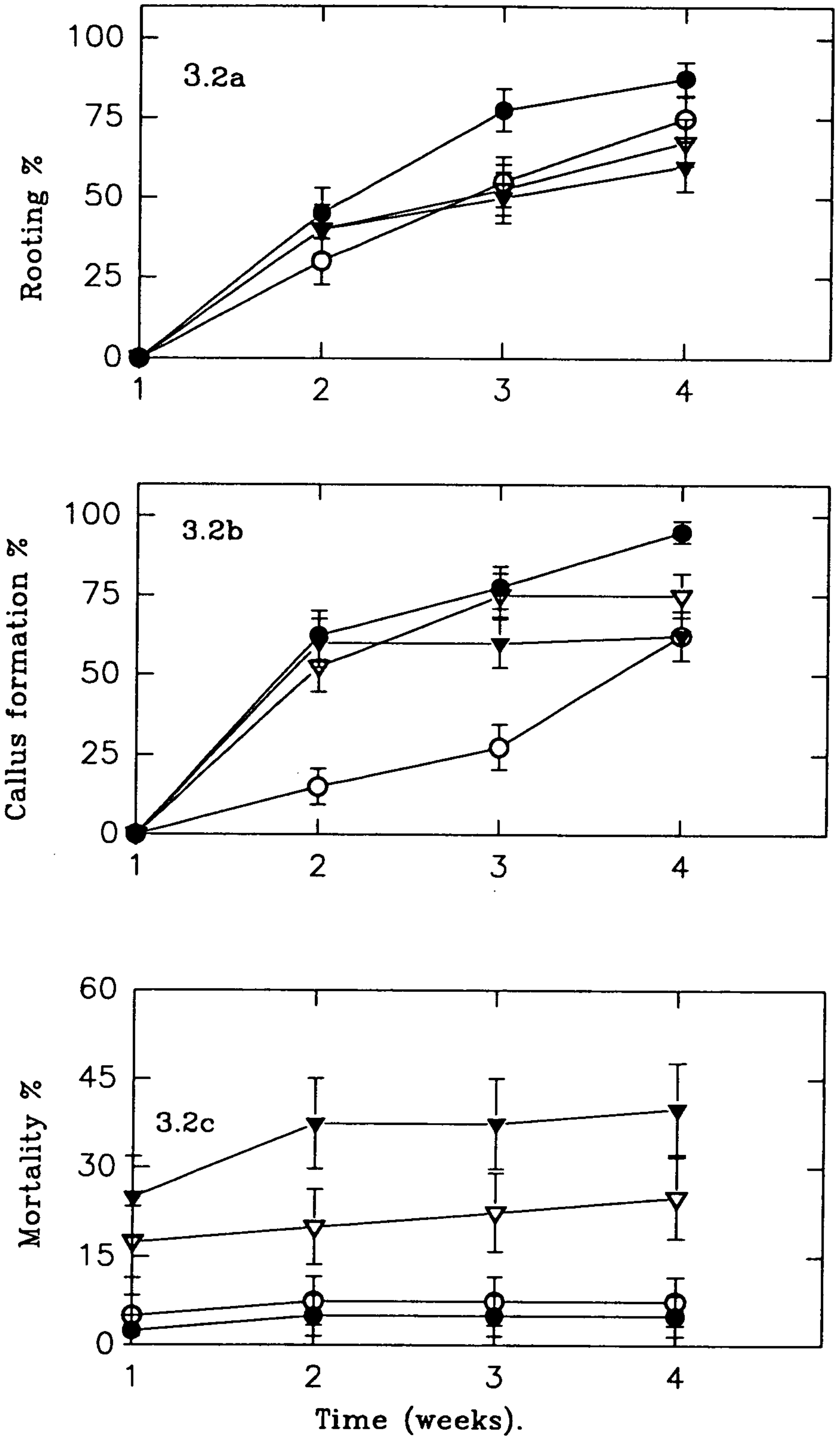
Number of roots per cutting

After the four weeks of this experiment, treatments 8, 40 and 200 μg IBA were found, in terms of mean root number per rooted cutting, to be significantly higher ($P < 0.05$; t-test) than the 0 μg treatment. Mean root numbers of 2.7, 6.4, 6.7 and 9.5 were recorded in 0, 8, 40 and 200 respectively (Figure 3.2f).

3.4 Experiment 3: Effects of different leaf areas on rooting of leafy stem cutting of *Ricinodendron heudelotii*

3.4.1 Introduction

The role of the leaf on adventitious root formation in cuttings appears to include hormonal, photosynthetic, water balance through stomatal conductance and gas exchange aspects of the rooting process. Leaves may enhance the uptake of applied auxin (Audus, 1963), and use some of the applied auxin to enhance substrate concentrations in the leaves such that the synthesis of endogenous auxins may be promoted (Middleton *et al.*, 1980). They then load the xylem of the cutting stem with


3.2a
Rooting %
100
75
50
25
0
1
2
3
4
3.2b
Callus formation %
100
75
50
25
0
1
2
3
4
3.2c
Mortality %
60
45
30
15
0
1
2
3
4
Time (weeks).

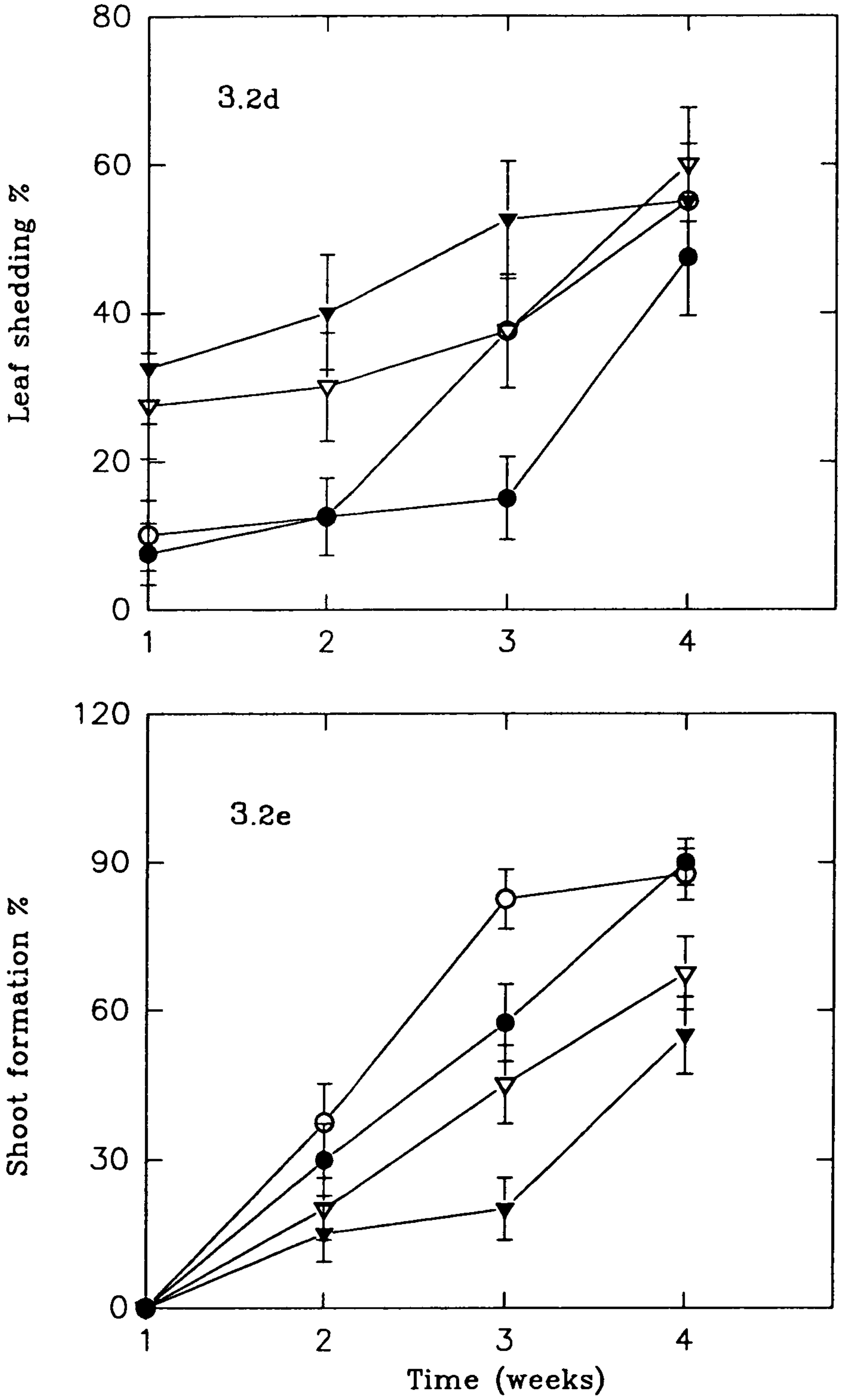


Figure 3.2: Effect of auxin (IBA) concentration on rooting of leafy stem cuttings of *Ricinodendron heudelotii* (○ = 0 μg, ● = 8 μg, ▽ = 40 μg and ▼ = 200 μg). Bar = ± SE. (n = 40).

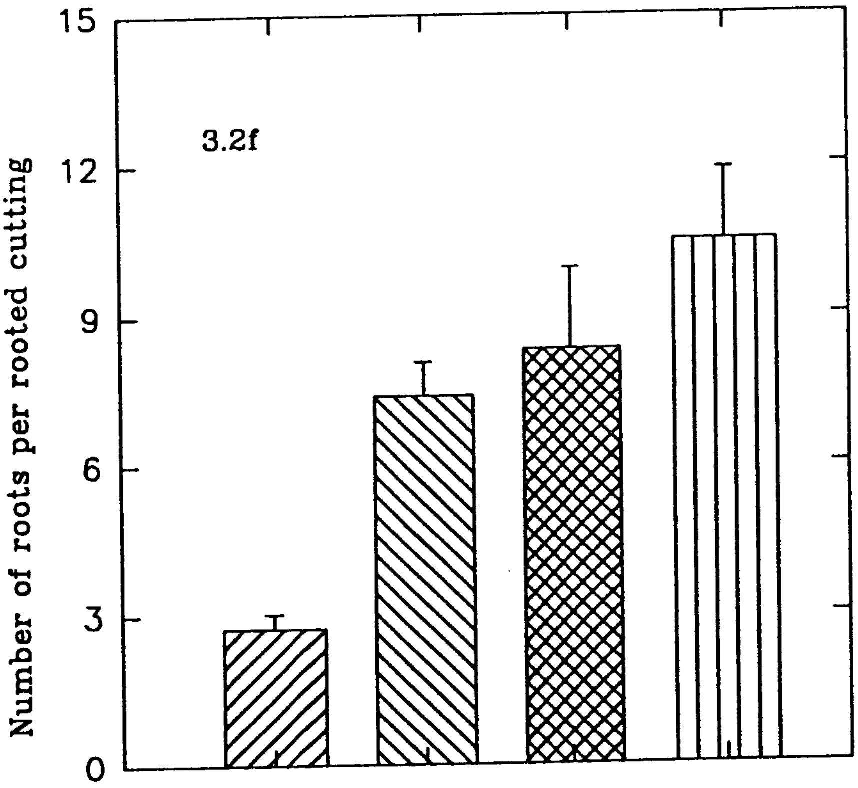


Figure 3.2f: Effect of auxin (IBA) concentration on root number per rooted cutting of leafy stem cutting of *R. heudelotii*. (▨ = 0 µg, ▧ = 8 µg, ▩ = 40 µg and ▥ = 200 µg; after 4 weeks). Bar + SE (n = 40).

auxins for transportation to the potential sites of root formation (Jarvis, 1986). Another contribution of leaves to the rooting process is photosynthesis, which often leads to accumulation of current photosynthates at the base of the cuttings (Reuveni and Raviv, 1981; Newton *et al.*, 1992b). It has also been found that water uptake through leaf surfaces during rooting of leafy stem cuttings contributes significantly to their water balance (Grange and Loach, 1983).

It has been observed that at high temperatures and low humidities, large leaf areas tend to be detrimental to rooting through high transpiration rates resulting in drought (Grange and Loach, 1983; Loach, 1977). Leakey *et al.* (1982b) reported that in *Triplochiton scleroxylon*, leaf areas of up to 900 cm^2 were space demanding, and could increase transpiration losses which tended to inhibit rooting. A 50 cm^2 leaf area was found to be optimum for rooting the cuttings of this species.

Many studies have demonstrated that varying leaf areas affect the rooting ability of cuttings of various species differently. Examples of such studies by Leakey *et al.* (1982), Asanga (1989), Tchoundjeu (1989), Leakey (1990) and Newton *et al.* (1992b) were described in Chapter Two. There is experimental evidence that most leafless cuttings of tropical tree species fail to root or do poorly. Hamzah (1992), for example, recorded 1.7% rooting for leafless and 86.7-100% for leafed cuttings of *Hopea odorata*. Newton *et al.* (1992b) recorded 0% rooting of leafless and over 80% rooting for 15 cm^2 leafed cuttings of *Terminalia spinosa* and Leakey *et al.* (1982b) recorded 9% for leafless and above 75% rooting for 50 cm^2 leafed cuttings of *Triplochiton scleroxylon.*

No research has been carried out to investigate leaf area effect on rooting leafy stem cuttings of *Ricinodendron heudelotii* The present experiment was carried out to determine the optimum leaf area in the leafy stem cuttings of this species, in order to produce maximum rooting percentage.

3.4.2 Materials and methods.

One hundred and twenty seedlings of *Ricinodendron heudelotii* were collected from the Southern Bakundu Forest Reserve on the 2nd March 1992. They were potted into small-sized (10 cm diameter and 18 cm depth) polythene pots and nursed in the propagation unit until each of them had produced eight to twelve leaves. Four cuttings were taken from each plant according to node position and four treatments were applied randomly in order to avoid treatment effects being confounded by clone. The cutting of the first top node of each shoot was discarded as it was found to be too soft for rooting. The four treatments were 0 cm^2, 25 cm^2, 50 cm^2 and 80 cm^2. These areas were obtained using graph paper templates. The treatments were repeated on four cuttings from four different plants giving a total of sixteen cuttings in one block. This was replicated thirteen times. Indole-3-butyric acid (40 μg) was applied to the base of each cutting as described earlier. The thirteen blocks were randomised in the same propagator containing sawdust as the rooting medium, giving a total of fifty-two cuttings per treatment (Plate 3.1). The cuttings were assessed as described earlier.

Analyses

Percentage rooting results were analyzed at the end of the experiment after transformation (arcsin $\sqrt{\%}$) of the data by analysis of variance using SAS (1980). Analysis of deviance was also used as described in earlier experiments.

3.4.3 Results

Rooting percentage

The cuttings that were initially leafless did not root and most of them rotted in the second week of the experiment. In terms of percentage rooting, there were significant differences between the three leafed treatments by week two when the proportion of cuttings that rooted increased with increasing leaf area. This trend was maintained in the third week, when rooting percentages in leaf area treatments 50 and 80 cm^2 were significantly ($P < 0.05$; t-test) higher (69% and 85% respectively) than in the 25 cm^2 leaf area treatment, which recorded 44%. By the fifth week, there were no significant differences between treatments 50 and 80 cm^2, and between treatments 25 and 50 cm^2 ($P > 0.05$; t-test). Analysis of variance test showed that percentage rooting was significantly higher ($P < 0.001$) in the 80 cm^2 than in the 25 cm^2 treatment (Table A5), but did not differ significantly from the 50 cm^2 treatment In general, percentage rooting was positively related to increasing leaf area (Figure 3.3a).

By applying analysis of deviance for stepwise regression, it was found that the rooting ability of the cuttings of this species was highly ($P < 0.01$) affected by node position and leaf area, but was not affected by block and stem length (Table A6).

Callus formation

Cuttings commenced callusing by the first week after insertion in all leafy treatments, with the highest proportion of callused cuttings (73%) registered in the largest leaf treatment (80 cm^2), while 36% of the cuttings in the 25 cm^2 treatment had callused. Treatment 80 cm^2 was significant higher than 25 cm^2 ($P < 0.05$; t-test) in the second, third and fourth weeks of the experiment, although there were no significant differences ($P > 0.05$; t-test) between treatments 25 and 50 cm^2 in terms of callus formation. However, each treatment maintaining a fairly constant proportion of callused cuttings from week two to four. None of the leafless cuttings callused (Figure 3.3b).

Cutting mortality

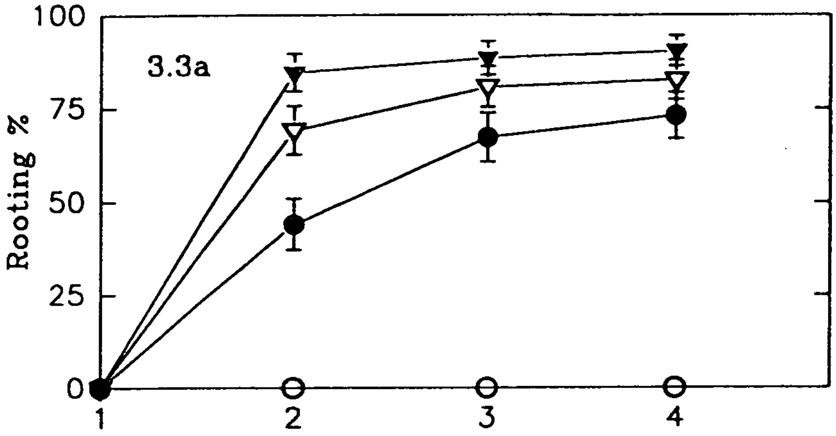
By week two, 55% of the leafless cuttings had died, and by the third week, mortality was 100%. At the end of week one, the proportion of dead cuttings varied from 3.8 to 15% in treatments 80 and 25 cm^2 respectively, and increased very slightly between the second and fourth weeks, when 17%, 12% and 8% mortalities were recorded in the 25, 50 and 80 cm^2 treatments respectively. These three treatments did not differ from each other significantly throughout the experiment (Figure 3.3c).

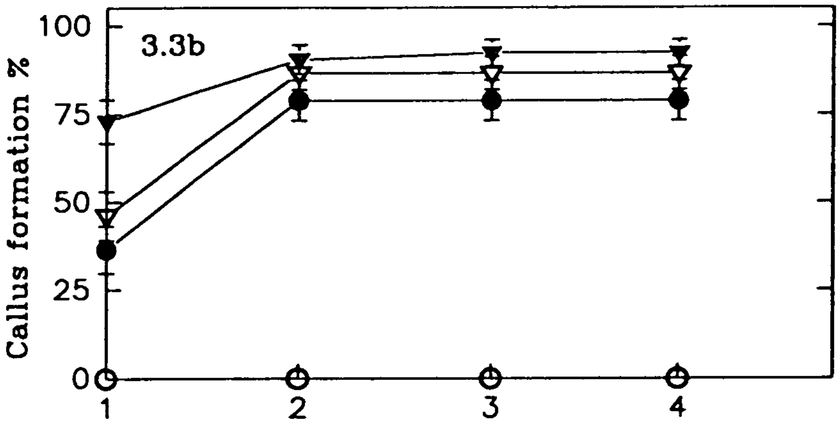
Leaf shedding

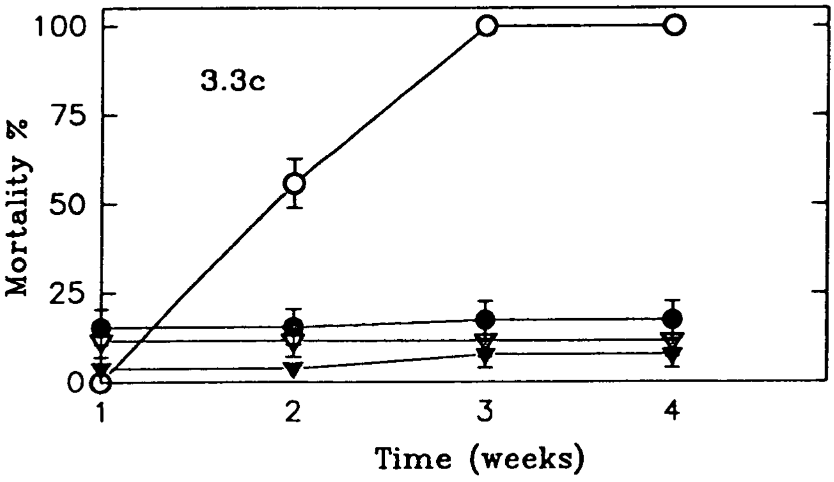
At the end of the first week after insertion, 15%, 12% and 4% of the cuttings in the leaf area treatments 25, 50 and 80 cm^2 respectively had shed their leaves. The proportion of leaf shedding in the three treatments increased to the second week, where all the treatments differed significantly from each other in this variable, with highest leaf shedding (15%) reached in the 25 cm^2 treatment. Between week two and three, leaf shedding in the 50 cm^2 leaf area treatment increased at a higher rate than in the 80 cm^2 leaf area treatment, such that treatments 50 and 25 cm^2 were significantly higher than the 80 cm^2 treatment in weeks three and four.

Shoot formation

New shoot formation differed significantly between the four treatments one week after inserting the cuttings, with percentage shoot formation (0, 0, 17%, and 42%) increasing with increasing leaf area (0, 25, 50 and 80 cm^2 respectively). Percentage shoot formation increased very rapidly in all the treatments, except the control, by week two, when the significant differences between the treatments were maintained. No significant differences (P >0.05; t-test) in the three leaf area treatments were observed in weeks three and four (Figure 3.3e).


3.3a
Rooting %
100
75
50
25
0
1
2
3
4


3.3b
Callus formation %
100
75
50
25
0
1
2
3
4


3.3c
Mortality %
100
75
50
25
0
1
2
3
4
Time (weeks)

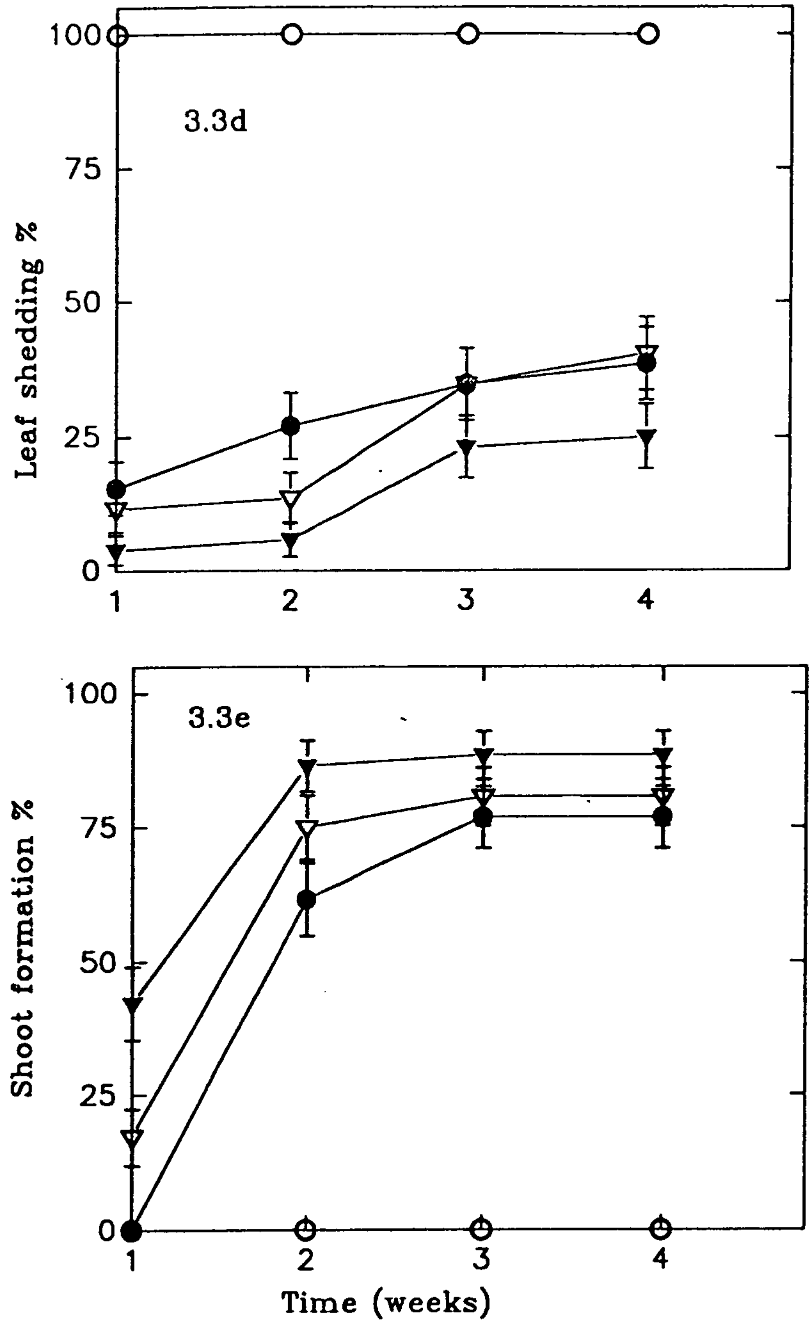


Figure 3.3: Effect of leaf area on rooting of leafy stem cuttings of *Ricinodendron heudelotii*, (○ = 0 cm^2, ● = 25 cm^2, ▽ = 50 cm^2 and ▼ = 80 cm^2). Bar = ± SE. (n = 52).

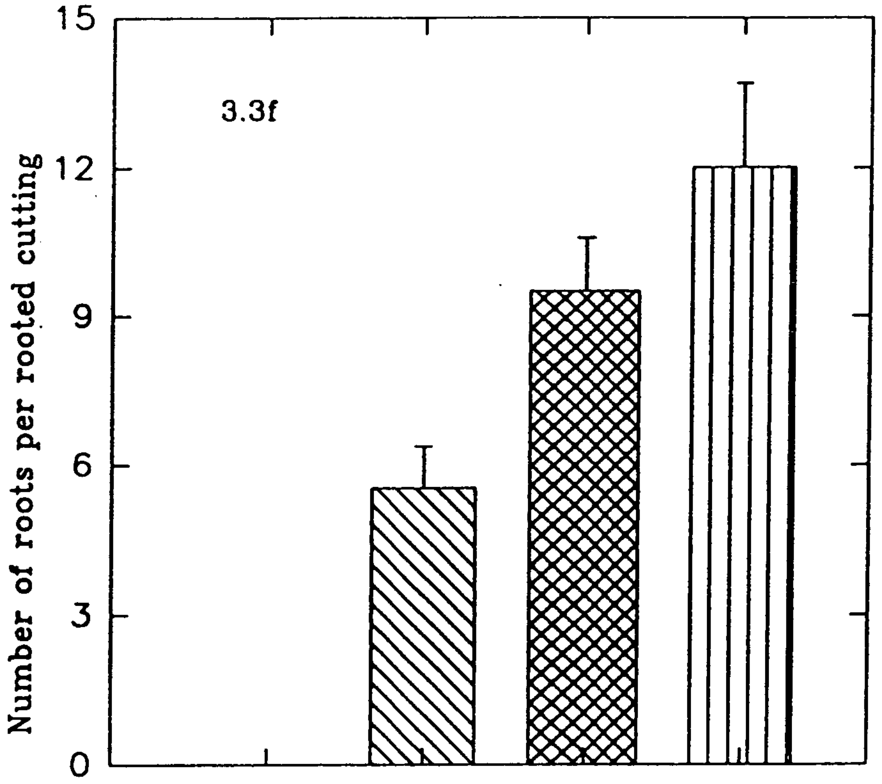


Figure 3.3f: Effect of leaf area on number of roots per rooted cuttings of *R. heudelotii*. ▧ = 25 cm^2, ▩ = 50 cm^2 and ▥ = 80 cm^2), (after 4 weeks). Bar = + SE. (n = 52).

Number of roots per cutting

Treatment 80 cm^2 was significantly higher ($P < 0.05$; t-test) than treatment 25 cm^2 in this variable after week four. The mean root number per rooted cutting did not differ significantly ($P > 0.05$; t-test) between treatments 80 cm^2 and 50 cm^2 and between 50 cm^2 and 25 cm^2. The control (0 cm^2) leafless cuttings did not root. At week four, mean root numbers 0, 5.6, 9.3 and 11.9 were recorded in treatments 0 cm^2, 25 cm^2, 50 cm^2, and 80 cm^2 of leaf area respectively (Figure 3.3f).

3.5 Discussion

Rooting percentage

In general terms, *Ricinodendron heudelotii,* as shown by the results of these three experiments, is amenable to low-technology vegetative propagation techniques developed for use in the tropics (Leakey *et al.*, 1990; Newton *et al.*, 1992a). The highest rooting percentage in experiments 1, 2 and 3 were 90%, 88% and 82% respectively, with the highest reached in the media experiment. However, in each of the experiments, rooting percentage was affected by the experimental treatments, indicating that a precise set of procedures should be used to optimise rooting.

In experiment 1, significant differences existed in the rooting ability of the cuttings of *Ricinodendron heudelotii* in the six different media tested. In this regard, this species exhibited a similar rooting response to other tropical tree species, such as *Cordia alliodora, Gmelina arborea, Vochysia hondurensis, Eucalyptus deglupta* and *Albizia guachapele* (Leakey *et al.*, 1990). In addition, cuttings of *Milicia excelsa* (Ofori, 1994), *Cordia alliodora* (Mesén, 1994), nine woody ornamentals (Loach, 1988) and *Lovoa trichilioides* (Tchoundjeu, 1989) also displayed significant differences in rooting ability in different media.

The results of this experiment showed that the highest rooting percentage (82%) was recorded in sawdust. This contrasts with results from other species, such as *Cordia alliodora*, where the lowest rooting percentage was recorded in sawdust (Mesén, 1994), *Gmelina arborea,* where highest rooting percentages were recorded in fine sand; *Vochysia hondurensis*, where highest rooting percentage was recorded in gravel; *Eucalyptus deglupta* and *Albizia guachapele*, where the optimum medium in terms of rooting percentage was a 50:50 mixture of fine sand and sawdust (Leakey *et al.*, 1990). However, in this latter investigation, sawdust was not tested separately. In addition, there are lots of variations in sawdust, such as particle size, the species from which it is produced, the age, colour and tannin content. The sawdust used in this study was rotted for more than six months and was produced from red wood as described earlier. The kind of sawdust used in these other experiments is not described, and could be different, thus causing the differences in rooting. In *Lovoa trichilioides*, 75% of the cuttings rooted in gravel and only 40% rooted in a 50:50 mixture of coarse gravel and forest topsoil (Tchoundjeu, 1989) and in four ornamental species, 69% and 43% of rooted cuttings were recorded in a 2:1 mixture of grit and peat and peat respectively (Loach, 1988). However, *Milicia excelsa* cuttings displayed the highest percentage rooting in sawdust, with the second highest in a 50:50 mixture of gravel and sawdust in an experiment testing four media (Ofori, 1994), while in a separate experiment, Mesén (1994) recorded 89% rooted cuttings of *Cordia alliodora* in gravel as against 76% in sawdust.

The reasons why different species display contrasting rooting percentages in different rooting media are not well understood (Leakey *et al.*, 1990). Rooting variation of cuttings can be attributed to the oxygen, mineral and water content as well as the pH and the porosity of the media, which affect tissue respiration in the process of cell dedifferentiation for root primordia production at the base of the cutting (Loach 1985; Haissig, 1986; Loach, 1988).

In Experiment 2, the effect of auxin (IBA) on the percentage rooting of *Ricinodendron heudelotii* cuttings was not significantly different to the control at week four (ANOVA) (Table A3). The fact that some cuttings rooted without IBA treatment indicates that this species is similar to *Lovoa trichilioides* (Leakey, 1994 pers. comm.), *Vochysia hondurensis* (Leakey *et al.*, 1990), *Shorea macrophylla* (Lo, 1985) and *Hopea odorata* (Hamzah, 1991), which were successfully rooted without auxin application. However, while rooting was completely absent in the control treatment (without IBA application) in *Cordia alliodora*, 20%, 45% and 55% of rooted cuttings were recorded in *Albizia guachapele, Vochysia hondurensis* and *Ricinodendron heudelotii* respectively without IBA. This suggests that auxin application is not a limiting factor in rooting the cuttings of *R. heudelotii* and some other species. The knowledge of such species is of practical importance as they may be propagated without the additional costs of auxin and labour required for auxin application.

However, the percentage of rooted cuttings treated with 8 μg of IBA was significantly higher than the percentage rooting of cuttings in the higher two IBA concentrations. Percentage rooting of the cuttings of this species decreased with increasing IBA concentration, with 88%, 68% and 60% rooted cuttings associated with 8 μg, 40 μg and 200 μg treatments respectively. These results contradict those of *Cordia alliodora, Albizia guachapele* and *Vochysia hondurensis,* where percentage rooting increased with IBA concentration up to an optimum value for each species and then declined with increasing auxin dose (Leakey *et al.*, 1990). Contrary to the above, the rooting percentage of *Debregeasia salicifolia* cuttings increased with increasing IBA concentrations of 100 μg, 200 μg and 500 μg associated with 30%, 35% and 40% respectively (Bhatt and Todaria, 1993).

Although the reasons for the different requirements with respect to auxin concentration for rooting of different species are poorly understood (Leakey *et al.*, 1990), they may be ascribed to the fact that endogenous auxin content of cuttings before severance varies with species. Endogenous contents may be above or below threshold values for rooting such that exogenous application can enhance or inhibit rooting to different degrees or have no effect on the cuttings. The influence of auxin on rooting of cuttings has been attributed to its effect in mobilizing carbohydrate reserves by enhancing the activity of hydrolytic enzymes (Nanda *et al.*, 1968b; Rana *et al.*, 1987). Middleton, Jarvis and Booth (1980) suggested that IBA enhances substrate concentrations in the leaves of fresh cuttings thereby promoting the synthesis of endogenous auxin, which is essential in root initiation in cuttings. Haissig (1986) suggested that the role of auxin in increasing stem respiration rate in cuttings has a promotive effect on rooting. Auxins may also conjugate toxic compounds and reduce or inhibit the synthesis of rooting inhibitors (Gaspar and Hofinger, 1988; Salisbury and Ross, 1978) such as gibberellin (Hartmann and Kester, 1983). Auxin can play a promotive role in the synthesis of enzymes and amino acid compounds as precursors in the eventual synthesis of ribonucleic acid and deoxyribonucleic acid (Salisbury and Ross, 1978), which carry coded genetic information during somatic cell division in the rooting process of cuttings.

The fact that none of the leafless cuttings in Experiment 3 rooted stresses the role that leaves play in rooting leafy stem cuttings of *Ricinodendron heudelotii.* This concurs with the results of Newton *et al.* (1992b) who found that, in a leaf area experiment with the cuttings of *Terminalia spinosa*, a multi-purpose tree species native to the semi-arid lands of East Africa, all the leafless cuttings failed to root. Breen and Muraoka (1974) also reported the failure of defoliated cuttings of a plum species (marianna 2624) to root even when treated with IBA, while control cuttings rooted when only half of the leaf was present. However, Leakey *et al.* (1982b) reported 9% rooting success of leafless cuttings of *Triplochiton scleroxylon* and 2% of *Hopea odorata* leafless cuttings have also been found to produce roots (Hamzah, 1992).

Although there were no significant differences in Experiment 3 between the treatments in terms of percentage rooting as far as the three leaved treatments were concerned, percentage rooting tended to increase with increasing leaf area. These results suggest that this species can root even with leaf areas larger than 80 cm^2. This contradicts the results of *Triplochiton scleroxylon* (Leakey *et al.*, 1982b) and *Khaya ivorensis* (Asanga, 1989; Tchoundjeu, 1989), where cuttings tended to maximize root percentage at leaf areas of 50 and 10-30 cm^2 respectively, and *Terminalia spinosa* (Newton *et al.*, 1992b) and such other tropical species as *Nauclea diderrichii* (Leakey, 1990), which displayed no tendency towards an optimum leaf area. The relationship between lamina area and percentage rooting of cuttings is of practical importance because the former can easily be manipulated by trimming. As far as the water economy of the cutting is concerned, transpiration losses can be reduced by trimming the leaf surfaces. The 900 cm^2 leaves of the cuttings of *Triplochiton scleroxylon*, for example, were trimmed to smaller areas (100, 50 and 5 cm^2) to decrease transpiration losses and because they tended to occupy too much space and were awkward to handle in the rooting medium (Leakey *et al.*, 1982b). Geary and Harding (1984) found that leaf trimming in *Eucalyptus camaldulensis* could enable the cuttings to occupy a much smaller bed area, reduce propagation costs, enhance cutting survival if the mist system failed, stimulate foliar bud development, and stimulate rooting. The actual wounding of the leaf in the process of trimming might have a part to play in promoting rooting and budbreak (Geary and Harding, 1984). Leaves may also contribute to the rooting process by photosynthesis leading to carbohydrate accumulation at the base of the cutting stem (Leakey and Coutts, 1989; Reuveni and Raviv, 1981; Newton *et al.*, 1992b). Carbon dioxide uptake and the process of transpiration, both take place in the leaf of the cutting (Dick *et al.*, 1994; Rhizopoulou and Diamantoglou, 1991; Newton and Jones, 1993b; Gay and Loach, 1977; Mesén, 1994). The balance between the process of photosynthesis and transpiration is thought to be responsible for the tendency of many species to display optimal leaf areas for rooting (Leakey and Coutts, 1989).

Callusing

The rate of percentage callus formation differed in the cuttings of the leaf area experiment from that in the cuttings of the auxin (IBA) and media experiments. Callus formation in cuttings did not start in the former experiment until week three, while it commenced in the latter two experiments in the second week after insertion (see Figures 3.1b, 3.2b, and 3.3b). The proportion of callused cuttings in experiment 3 was higher (92%) than that in experiment 1 (90%) and in experiment 2 (78%) in the third week.

The highest proportion of callused cuttings in Experiments 1, 2 and 3 were 98%, 95% and 92% respectively, with Experiment 1 having the highest percentage callus formation. The highest proportion of cuttings that callused in the three experiments were associated with sawdust, 8 μg of auxin (IBA) and 80 cm^2 of leaf area. This suggests that if a combination of these treatments is applied to the cuttings of this species, percentage callus formation may be maximized.

In experiment 1, the highest proportion of cuttings that callused was associated with the sawdust rooting medium. These results showed that most of the cuttings which formed callus eventually rooted, although the amount of callus on an individual cutting appeared not to be related to whether or not the cutting rooted. This observation is consistent with that of Hartmann and Kester (1983), who indicated that in general, callus formation in cuttings of most species is independent of rooting and with the view of Van der Lek (1925, cited by Komissarov, 1969) that root formation in cuttings is not connected with the activity of callus. Newton and Jones, (1993a) also reported that many cuttings of *Cordia alliodora* produced callus at the base of the stem but failed to root.

The role of callus in rooting cuttings is poorly understood because the physiology of callus has not been sufficiently studied (Komissarov, 1969). However, it has been thoroughly studied in tissue culture. It is believed that callus enhances the supply of water to cuttings during rooting in the rooting media (Flerov and Kovalenko 1952; Buryi, 1901, cited by Komissarov, 1969). However, excessive callus formation may inhibit the initiation of adventitious roots. This concurs with the observation in the present study where cuttings with massive callus did not root at all or took a longer time to root. However, it has been shown that callus cell masses decrease with increasing alkalinity (pH) of the medium (Hartmann and Kester, 1983). This suggests that at high pH, a medium may be detrimental to rooting. The pH of the medium could, therefore, be adjusted to reduce callus and enhance root production (Park and Andersen, 1989; Hartmann and Kester, 1983).

In experiment 2, the treatment in which the highest percentage rooting was registered (8 μg) also reached highest callus formation. However, in the other treatments, percentage rooting was not closely linked with callus formation. This is in contrast to the findings of Uniyal *et al.* (1993), who reported that although callus formation was seen on cuttings of all treatments, further differentiation into roots was achieved only in auxin treated cuttings of *Dalbergia sericea*. Furthermore, while a high proportion of the untreated cuttings of *Ricinodendron heudelotii* callused and rooted, those of *Dalbergia sericea*, which did not receive any auxin (control) treatment, all callused, but completely failed to root. The non-differentiation of callus into roots has been attributed to lack of sufficient nutrient reserves, internal factors or the age of the cutting (Bonga and Durzan, 1982).

The leafless cuttings in Experiment 3 failed to callus. However, callusing results of the leaved treatments showed a similar pattern to those of percentage rooting, with the highest leaf area (80 cm^2) having the greatest proportion (92%) of callused cuttings.

Mortality

Cutting mortality started in all the three experiments one week after cutting insertion, with the highest percentage mortality (in each case) of 10%, 25% and 15% recorded in experiments 1, 2 and 3 respectively. By the end of each of the three experiments, the proportion of dead cuttings ranged from 13% to 38% in experiment 1, 5% to 40% in experiment 2 and 8% to 100% in experiment 3 (Figure 3.1c. 3.2c and 3.3c). The lowest percentage cutting mortality in experiment 1 was recorded in the sawdust treatment, that in experiment 2 in the 8 μg treatment and that in Experiment 3 was registered in the 80 cm^2 leaf area treatment. This observation contradicts that of Mesén (1994), who recorded the highest percentage of rotted cuttings of *Cordia alliodora* in sawdust. In the present study, the highest percentage rooting was registered in this medium, indicating an inverse relationship between percentage cutting mortality and percentage rooting.

The cuttings of this species died by rotting, which either started from the basal end in the rooting medium or from the top, after absessing the leaf. Rotting at the cutting base in the medium may be attributed to waterlogging (O'Dell and Stoltz, 1978), particularly after leaf abscission, when transpiration is arrested. This may lead to inadequate supply of oxygen for tissue respiration, which may in turn arrest starch interconversion and carbohydrate metabolism to release energy for mitosis and cell de-differentiate to form primordia from which cells differentiate into root initials (Leakey *et al.*, 1993; Dick and Dewar, 1992). It is also known that in waterlogged media, inadequate oxygen supply is due to the slow rate at which it diffuses through water (10,000 times slower than in air) (Loach, 1986). Basal cutting mortality could also have been caused by mechanical blockage of the sieve tubes of the xylem cells by gummy materials of carbohydrate origin and callus formation (Mujib, 1993), tiny sand and sawdust particles. Rotting at the cutting top could be ascribed to a pathological attack resulting from a bacterial and/or mycotic infection. The internodes of some single-node cuttings were not long enough to reach the water table.

Consequently, they may have desiccated because of a lack of water supply. However, leaf loss was not always related to cutting mortality. Some cuttings did not die after leaf abscission and vice versa.

The highest percentage cutting mortality of leaved cuttings in the mixture of gravel and sawdust treatment could have been caused by the low air:water ratio of 0.20 ± 0.02, with a composition of 7.2% air, 36.0% water and 56.8% solid. In this medium, the percentages of air and water were low, compared to those in sawdust where the highest proportion of rooted cuttings was recorded. The low percentage of air could inhibit tissue respiration and carbohydrate hydrolysis at the base of the cuttings and this could cause cutting mortality in this medium (Dick *et al.*, 1994). The second highest cutting mortality was recorded in the medium sand treatment which had a much lower air:water ratio than the mixture of gravel and sawdust medium. The water content of this medium is equally high as in the mixture. This could have accounted for the high mortality in this rooting medium. This could have led to waterlogging resulting in anoxia within the tissues of the cuttings (Loach, 1986). The observation that many cuttings died by rotting is consistent with this hypothesis.

In experiment 2, most of the cuttings which did not survive in all the four treatments died in the first week of the experiment. The highest cutting mortality of 40% was recorded in the highest auxin (200 μg IBA) concentration, and the lowest (5%) was associated with the lowest hormone concentration (8 μg) which was associated with the highest percentage rooting. Mortality increased very slightly from week two to four. Except for the control treatment, cutting mortality varied inversely with IBA concentration.

The fact that cutting mortality was negatively related to percentage rooting suggests that at higher doses, IBA might have become toxic to the cuttings. Middleton *et al.* (1978) reported that high doses of auxin induced phytotoxicity in the cuttings of mung bean seedlings. The toxicity of IBA at high concentrations could have hindered the activities of the hydrolytic enzymes and reduced auxin effect in mobilizing carbohydrate reserves to the rooting zone (Nanda *et al.*, 1968b; Rana *et al.*, 1987), resulting to cutting mortality. High doses of IBA might have inhibited substrate concentrations in the leaves and stem respiration rate thereby hindering the synthesis of endogenous auxin and life processes (Middleton, Jarvis and Booth, 1980; Haissiig, 1986), all of which are detrimental to the cuttings.

In experiment 3, cutting mortality tended to increase with decreasing leaf area, from weeks three to four, with the largest (80 cm^2) leaf area having the lowest proportion (8%) of dead cuttings, while the leafless cuttings were all dead (Figure 3.3b). The fact that none of the leafless cuttings died in week one while 15% of the leaved cuttings were dead, suggests that the leafed cuttings might have suffered from water stress resulting from high transpiration rate (Grange and Loach, 1983).

The negative relationship between cutting mortality and increasing leaf area in this experiment may be ascribed to the fact that current photosynthate production was likely to be higher in the larger leaf areas than in the smaller ones, although it was not measured. This could have provided more current assimilate for respiration, and perhaps reduced the incidence of cutting mortality in this way.

Leaf abscission

In all the three experiments in this Chapter, percentage leaf abscission commenced in the first week after cutting insertion. At this time, (percentage) leaf abscission ranged from 3% to 30% in experiment 1, 8% to 32% in experiment 2 and 3.8% to 15% in experiment 3. The lowest percentage leaf shedding in experiment 1 was recorded in the fine sand treatment, in experiment 2 it was in the 8 μg treatment and in experiment 3, it was in the 80cm^2 treatment.

Very few cuttings in sawdust lost their leaves in experiment 1, when the highest proportion of leaf abscission was registered in the gravel:sawdust medium, which had the lowest proportion of rooted cuttings of this species. Highest leaf abscission was reached in the first week. Similarly, leaf abscission in most of the cuttings in experiment 2 started in week one. Although there was no significant difference between the treatments with respect to leaf abscission, the greatest proportion of cuttings that shed their leaves was associated with the 40μg treatment. Interestingly, this is the treatment in which the highest rooting percentage and percentage callus formation were registered. In this regard, it was observed that leaf abscission was lowest in the treatments where the percentage of rooted cuttings was highest.

In experiment 3, the lowest proportion of leaves that absessed was associated with the largest leaf area. This contradicts the results of Newton *et al.* (1992b), who observed in a leaf area experiment with *Terminalia spinosa*, that a greater mean proportion of leaves were shed by cuttings with larger leaf areas. Overall, leaf abscission was negatively related to leaf area in *R. heudelotii*. Asanga (1989) obtained contrary results with *Khaya ivorensis* cuttings, where higher percentage of leaved cuttings with larger leaf areas abscessed leaves than cuttings with smaller leaf areas. Leaf abscission appeared to be more associated with senescence than with treatments since mostly the leaves of cuttings from the lower node positions abscised. In some cases, shed leaves were replaced by shoots which could take over the functions of the abscised leaves, particularly in the synthesis of auxin (Hartmann and Kester, 1983). However, in other cases, leaf abscission was associated with cutting death.

Shoot outgrowth

The time of shoot formation by cuttings differed in the three experiments in this chapter. While a maximum proportion of 2% and 42% of the cuttings in experiments

1 and 3 respectively formed shoots in the first week after insertion, the cuttings in experiment 2 did not form shoots until week two. (see Figure 3.1d, 3.2d and 3.3d).

In experiment 1, the rooting media which enhanced rooting and callus formation, also favoured new shoot formation such that the cuttings in sawdust produced the highest proportion of new shoots. Shoots produced by cuttings while in the propagation media have been found in some species to constitute a sink for nutrients to the detriment of root production (Haissig, 1986; Newton *et al.*, 1992a).

The results of experiment 2 showed that the treatments which enhanced percentage rooting again promoted new shoot formation, although cuttings in all treatments produced new shoots. This is in agreement with the results of Uniyal *et al.* (1994) who found that new shoots appeared on all cuttings of *Dalbergia sericea* irrespective of auxin concentration treatment. However, a lower proportion of sprouts appeared on cuttings treated with higher auxin concentrations than on those treated with lower concentrations. This is not consistent with the results of Uniyal *et al.* (1994), who found that in an auxin (IBA) experiment, 53% of the cuttings of *Dalbergia sericea* treated with 100 *ppm* sprouted as against 3% treated with 500 *ppm*.

The speed of new shoot formation in cuttings after insertion in the rooting media depends on the nutrient reserves available in them (Wright, 1975). The effect of leaf trimming has also been suggested to play a promotive role in budbreak and shoot formation in cuttings (Geary and Harding, 1984). Experimental evidence in more recent studies suggest that leaf regeneration during the rooting process of cuttings is at least partly dependent on the current production of assimilates (Newton *et al.*, 1992b).

At the end of experiment 3, all the leafless cuttings had died but the proportion of cuttings that formed new shoots in treatments 25, 50 and 80 cm^2 did not differ significantly from one another before this time. This is contrary to the findings of Newton *et al.* (1992b), who found that the leafless cuttings of *Terminalia spinosa* sprouted and produced new leaves. In this experiment, it was found that the proportion of cuttings that sprouted was positively related to lamina area. This is consistent with the results of Geary and Harding (1984), who reported a positive relationship between flushing percentage and increasing number of leaves retained on the cuttings of *Eucalyptus camaldulensis.* These findings are in agreement with the experimental evidence that cuttings with leaves are photosynthesizing during propagation (Newton *et al.*, 1992b; Leakey and Coutts, 1989). The current assimilates resulting from it may be vital in the production of roots, buds, shoots and leaves in cuttings, with larger amounts being produced by the larger leaf areas in this experiment.

Root number

In experiments 1, 2 and 3, mean number of roots per rooted cutting ranged from 3.6 to 7.3, 2.8 to 10.5 and 0 to 12.1 respectively, with the zero value being associated with the leafless treatment in experiment 3. The highest mean number of roots per rooted cutting was associated with the sawdust treatment in experiment 1, the 200 μg of IBA treatment in Experiment 2 and the 80 cm^2 leaf area treatment in experiment 3. Except for the IBA treatment in experiment 2, this is consistent with the observations on callus formation, where the highest percentage of in callus formation was registered in the sawdust, 8 μg (IBA) and 80 cm^2 leaf area treatments in experiments 1, 2 and 3 respectively.

Cuttings in the medium where the highest percentage rooting was recorded also gave the highest mean number of roots per rooted cutting, with sawdust and fine sand being highest and second highest respectively in this variable. This observation concurs with that of Ofori (1994), who recorded the highest mean number of roots per rooted cutting and the highest rooting percentage of *Milcia excelsa* in sawdust in a media experiment. The higher mean number of roots per cutting, the higher are the chances of the cuttings becoming successfully established when transplanted. The lowest mean number of roots per rooted cutting *R. heudelotii* was associated with gravel. This agrees with *Milicia excelsa* which produced the lowest mean number of roots per rooted cutting in gravel (Ofori, 1994).

In this experiment, the high number of roots per rooted cutting could be attributed to the high percentage of oxygen in the sawdust medium. This concurs the observation of Hartmann and Kester (1983), who reported that root production in carnation and *Chrysanthemum* cuttings increased markedly when water in which they were rooted was aerated with increasing amounts of oxygen, from 0 to 21%. The number of roots per rooted cuttings can be adversely affected by poor aerated, waterlogged, low moisturised and large (<6 mm in diameter) particle sized medium (O'Dell and Stoltz, 1978), such as gravel in the experiment described here.

In experiment 2, a definite trend of increasing root number with auxin concentration was displayed. This is consistent with the results of Leakey *et al.* (1982b) who demonstrated that the number of roots per rooted cutting of *Triplochiton scleroxylon* increased with increasing auxin concentration, and Bhatt and Todaria (1993) who observed the same tendency in the cuttings of *Debregeasia salicifolia* and *Bohemeria rugulosa* collected from altitudes of 1760 *m* and treated with different IBA concentrations, except the control where no cutting rooted. These findings, however, contrast with the results of Uniyal *et al.*, (1994) who found that the mean number of roots per rooted cutting at a lower concentration of IBA was more than double that at higher concentration. Mean number of roots per rooted cutting was inversely related to rooting percentage in the present experiment, with high auxin concentrations associated with slightly lower proportions of rooted cuttings and high number of roots per rooted cutting. A compromise has to be established between high percentage rooting and high root number per rooted cuttings in order to achieve large scale cutting production.

In experiment 3, the mean number of roots per rooted cutting increased with increasing leaf area, while all the leafless cuttings had died (Figure 3.3f). This is consistent with Breen and Muraoka (1974) who demonstrated that mean number of roots per rooted cutting of "Marianna 2624" plum (*Prunus cerasifera* and *Prunus munsoniana*) increased with increasing leaf number. It would appear that the leaf area above which the number of roots per rooted cutting may start to decline is greater than 80 cm^2.

CHAPTER 4

Effect of different media, IBA concentration, and leaf areas on rooting leafy stem cuttings of *Irvingia gabonensis*

CHAPTER 4

Effect of different media, IBA concentration, and leaf on rooting leafy stem cuttings of *Irvingia gabonensis*

4.1 Introduction

Although *Irvingia gabonensis* has been successfully budded (Okafor, 1971), no attempt to propagate this species by leafy stem cuttings has been reported previously. Owing to its importance in Cameroon (as highlighted in chapter one), and in West Africa in general (Okafor and Lamb, 1994), information on how to propagate this species successfully is of great value for enabling genetic improvement.

The experiments in this chapter investigated the influence of vegetative propagation by leafy stem cuttings on the rooting ability of *Irvingia gabonensis*. The study was undertaken by examining the effects of rooting media, auxin (IBA) concentration and leaf area on the rooting of cuttings of this species. The experiments were aimed at characterizing the treatments that would optimize rooting for future mass production of clonal planting stock.

4.2.0 Experiment 1: Effect of media on rooting of leafy stem cuttings of *Irvingia gabonensis*.

4.2.1 Introduction

The importance and influence of rooting media in root initiation and development in leafy stem cuttings were described in chapter three. This experiment was designed to determine the media that would maximize root formation in cuttings of *Irvingia* *gabonensis*. The treatments included SD, FS, G:SD, MS, G and MS:SD, all prepared as described in chapter two.

4.2.2 Material and methods

In mid February 1991, tree prospection tours were made to the Southern Bakundu Forest Reserve and nearby farms under the guidance of forest guards and farmers. Some phenotypically plus trees of *Irvingia gabonensis* (the sweet bush mango variety) were selected based on their superior fruiting habits, fruit and kernel size and fruit and seed taste according to information obtained from the guides. Although most of the trees on farms met these criteria, selection could not be made here because the farmers were not willing to spare their trees. For this reason, tree selection was limited to the Reserve where ten of the selected trees were numbered and stumped in the middle of March, 1991. The size of these trees made the original proposal to cut these stumps at 0.3 m inappropriate. Generally, 1m was used, although to get above the buttresses of some *Irvingia gabonensis*, heights in excess of this were necessary.

After about six months, the stumps produced sprout shoots. On the 24th of March, 1992, ninety single-node cuttings, five from each shoot, were harvested from each of four stumps numbered 1003, 1007, 1008 and 1012. After trimming the leaves to about 50 cm^2 (Leakey *et al.*, 1982b), the cuttings were treated with 200 μg of indole-3-butyric acid (IBA) in a drop from a micrometer syringe applied to the clean-cut base of each. They were then allowed to dry in a current of air from a fan. Fifteen cuttings from each clone were randomly selected and inserted, according to node position, into each of the six media. When the experiment was completely set, there were 60 cuttings in each medium (5 cuttings x 3 shoots per clone x 4 clones). Cutting lengths varied between 5 and 9 cm. The cuttings were sprayed with systemic insecticide and fungicide according to the instructions of the manufacturer. To keep the leaves wet, the cuttings were sprayed with pure water twice a day, before 7.30 and after 17.30 hours.

Assessment for callus and root formation, leaf shedding, shoot formation, cutting mortality, root diameter and cutting top and bottom diameters, were made as described earlier.

Analysis

The six compartments containing the different media were placed in the same propagator. Analysis of variance was applied to assess differences between the six rooting media, followed by Fisher's t tests (LSD) at the end of the experiment in week five using SAS (1980) as described earlier. In order to identify and understand the factors that were affecting the rooting ability of the cuttings of this species the most, analysis of deviance by stepwise regression in Genstat 5 (Payne *et al.*, 1987) was also employed on the other factors affecting the rooting ability of *I. gabonensis* at the end of the experiment in week five as described in early experiments.

4.2.3 Results

Percentage rooting

At the first assessment after week one, no cutting had rooted. Percentage rooting increased rapidly after week two and reached a maximum by week five. The percentage rooting of cuttings in SD was significantly higher than those in other treatments from week two onwards. Analysis of variance showed that FS was significantly higher ($P < 0.001$) than the other four media, which did not, in turn, differ significantly ($P > 0.05$) from each other at week five (Table A7). The lowest rooting percentages at week 5 (46% and 47%) were recorded in MS:SD and MS respectively (Fig.4.1a).

The data were analyzed by stepwise regression. It demonstrated that the rooting ability of the cuttings of *I. gabonensis* was significantly affected by media, node position and stem diameter at the base, but was not significantly affected by block and stem length (Table A8).

Callus formation

No cutting callused in week one in any of the six media. By week two callus formation had taken place in all the media, with percentages of callused cuttings ranging from 18% to 44% in G and G:SD respectively. This rose sharply to the third week, when the lowest value of 71% was associated with MS and the highest percentage callus formation of 100% was recorded in SD and MS:SD each. These two media were significantly higher in this variable than the other four, which did not differ significantly from each other. Between week three and four, the percentage of callused cuttings increased so rapidly in FS that it converged with that of cuttings in MS:SD and SD at the 100% locus, where these three media were significantly higher than the others. At week five, there were no significant differences between the six media in this parameter (P >0.05; t-test) (Figure 4.1b).

Cutting mortality

In the first three weeks, no cutting died in any of the media. By the end of week four, 6%, 4% and 4% of the cuttings had died in G, G:SD and MS respectively, while no cutting mortality was recorded in SD, MS:SD and FS. At the final assessment at week five, the proportion of dead cuttings in G, G:SD and MS (13%, 9% and 10.9% respectively) was significantly higher than it was in FS (2%), SD (0%) and MS:SD (0%) (Figure 4.1c).

Leaf shedding

Cuttings started to shed their leaves after three weeks in two media, namely MS and G:SD, where the leaves of 4% of the cuttings in each case had been abscessed. Leaf loss percentage increased to the fourth week when the proportion of cuttings without leaves was significantly greater ($P < 0.05$; t-test) in MS, G:SD, MS:SD and G than it was in the other three media, which were in turn not significantly different from each other. By week five, however, leaf shedding percent was significantly higher in G than it was in the other five media, and that in G:SD, MS and MS:SD was significantly higher than that in FS and SD. But there were no significant differences between G:SD, MS and MS:SD, and between SD and FS ($P > 0.05$; t-test) (Figure 4.1d).

Shoot formation

At week two, the formation of new shoots started in SD, MS:SD, MS and FS, where new shoots on 2% of the cuttings were recorded in each of the first three media and 6% in FS. Percentage shoot formation then increased sharply to week three, when SD was significantly higher ($P < 0.05$; t-test) than the other media except FS. However, FS was significantly higher than G. By the fourth week, G was significantly lower than the other five media. Percentage shoot formation then rose more sharply to the fifth week, when there were no significant differences ($P > 0.05$; t-test) between G and MS, and between SD and FS, although FS peaked (Figure 4.1e). At the end of the experiment in week five, percentage shoot formation in cuttings of 32.7%, 42%, 51%, 58%, 71% and 76% were recorded in G, MS, MS:SD, G:SD, SD and FS respectively (Figure 4.1e).

Number of roots per rooted cutting

After five weeks, the number of roots per rooted cutting (7.8) was significantly higher in SD than in the other five media ($P < 0.05$; t-test) (Figure 4.1f). In FS, the number of roots per rooted cutting (3.4) was significantly higher than in the rest of the treatments other than SD; there were no significant differences among the other media. The smallest number of roots per rooted cutting (1.8) was recorded in gravel.

4.3 Experiment 2: Effect of hormone (IBA) on rooting leafy stem cuttings of *Irvingia gabonensis*.

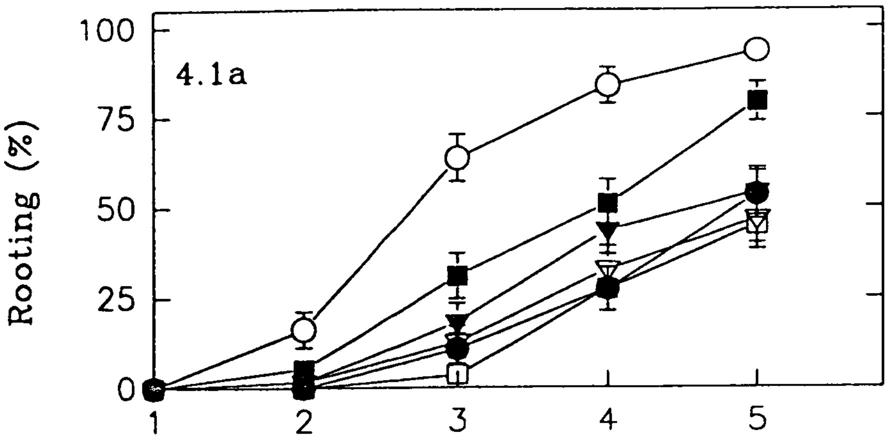
4.3.1 Introduction

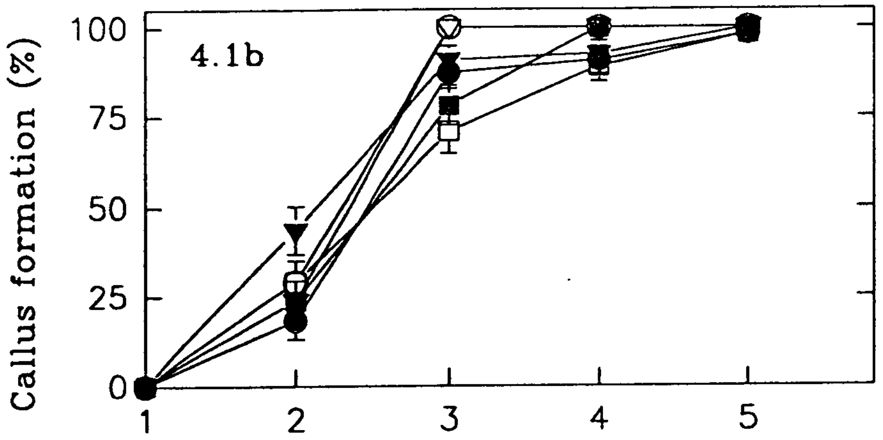
It is well established that growth regulators play a significant role in controlling the rooting ability of stem cuttings (Nanda, 1977; Leakey *et al.*, 1982b; Rana *et al.*, 1987; Uniyal *et al.*, 1993). However, rooting ability varies with species, different kinds of growth regulators and with different concentrations of individual auxins (Negi, 1982; Puri and Shamet, 1988).

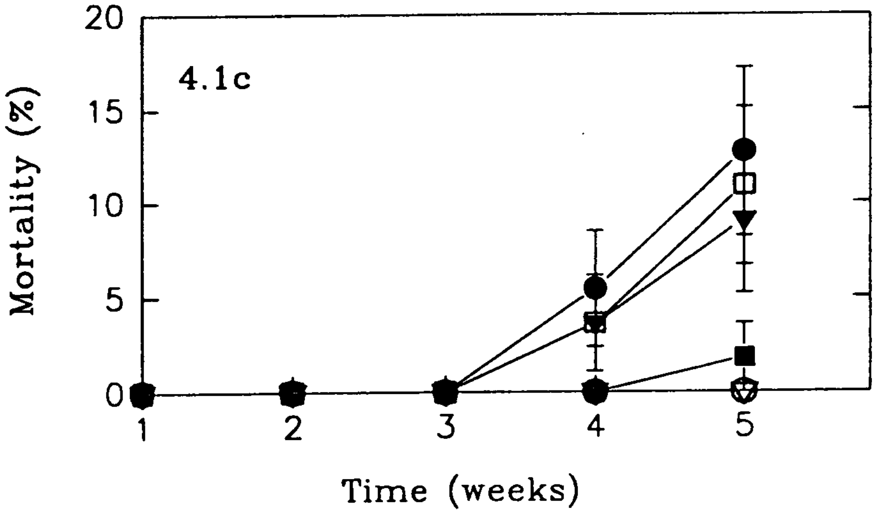
In the present study, the effect of variation in exogenous IBA application on the rooting of leafy stem cuttings of *Irvingia gabonensis* was studied. The treatments were 0 μg, 8 μg, 40 μg, 200 μg and 250 μg IBA applied per cutting; with the zero as a control. The aim was to determine the most suitable concentration for mass production of rooted cuttings of this species.

4.3.2 Materials and methods

Fruits of *Irvingia gabonensis* were collected from plus trees during a fruit hunt with village collectors from Tombel village. After the pulp rotted and freed the seeds,


4.1a
Rooting (%)
100
75
50
25
0
1
2
3
4
5


4.1b
Callus formation (%)
100
75
50
25
0
1
2
3
4
5


4.1c
Mortality (%)
20
15
10
5
0
1
2
3
4
5
Time (weeks)

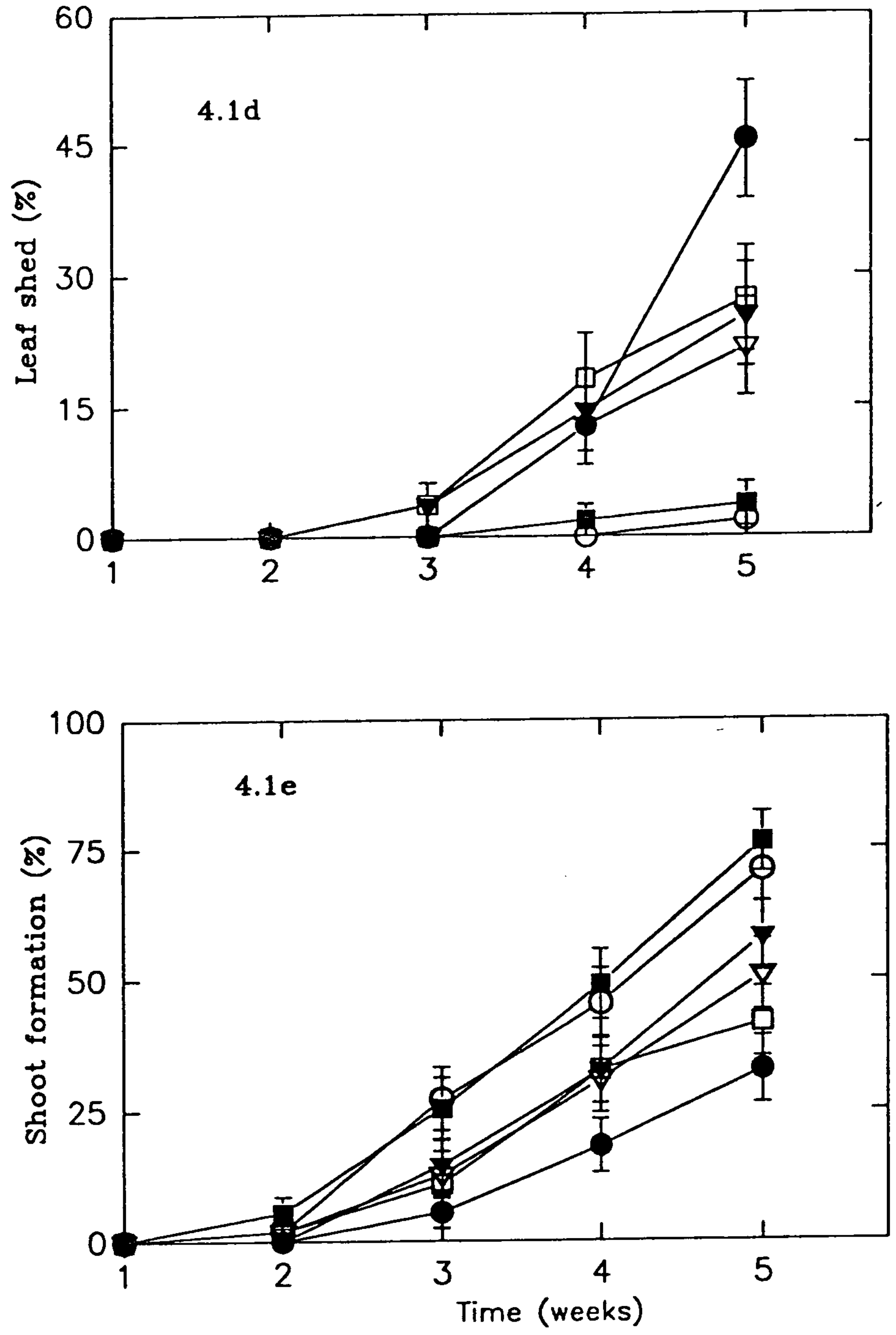


Figure 4.1: Effects of different media on rooting of leafy stem cuttings of *Irvingia gabonensis*.(○ = SD, ■ fine sand, ● = G, ▼ = G:SD, ▽ = MS:SD and □ = MS) Bar = ± SE. (n = 55).

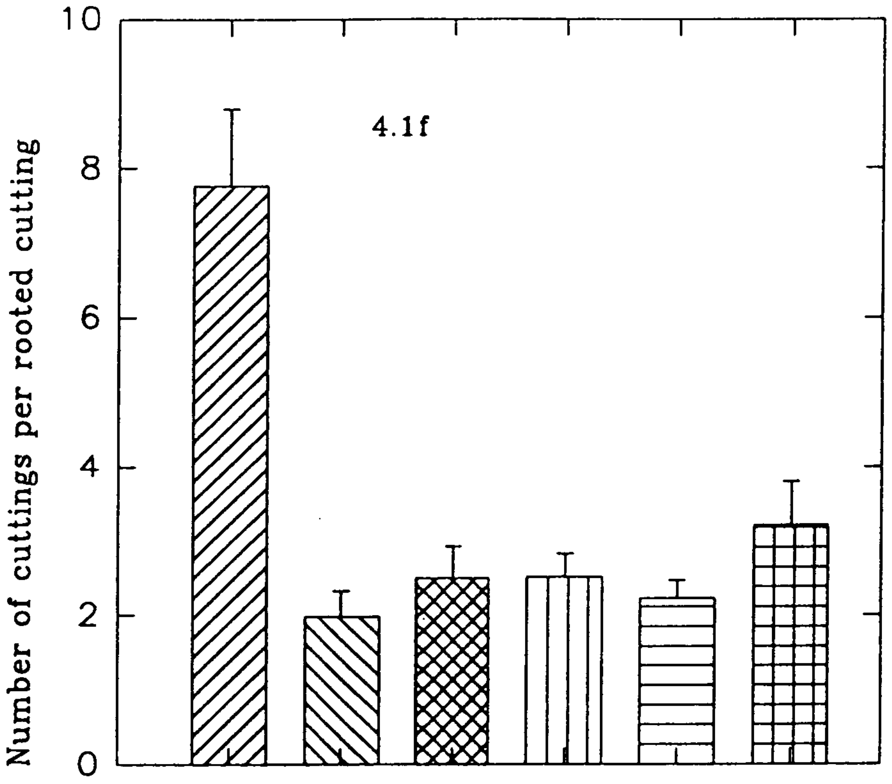


Figure 4.1f: Effect of media on number of roots per rooted cuttings of *I. gabonensis*. (▨ = SD, ▧ = G, ▩ = MS:SD, ▥ = G:SD, ▤ = MS and ▦ = FS). Bar + SE. (after 5 weeks). (n = 55).

they were dried in the sun for three days and sown on the 28th of July 1991. After germination, they were potted in medium-sized polythene pots (18 cm diameter, 26 cm depth) and nursed in the propagation unit. When the seedlings attained the height of about one meter, five cuttings were taken from each of five shoots, according to node position, on the 18th of April 1992. Five treatments were randomly applied to the five cuttings from the same shoot in order to avoid treatment effect being confounded with clonal variation after trimming the leaf to about 50 cm^2. This was repeated on five different shoots, giving a total of twenty-five cuttings per block. The five doses of indole-3-butyric acid (IBA) were applied as described in Chapter Three, with a drop of alcohol alone as a control treatment. There were ten blocks randomly set in the same propagator containing sawdust as the rooting medium (Plate 4.1). After two weeks the cuttings were assessed as described in early experiments.

Analysis

At the end of the experiment in week six, an analysis of variance and that of deviance were carried out to determine the effect of auxin (IBA) and that of the other factors investigated in this experiment on the rooting ability of this species as described earlier.

4.3.3 Results

Rooting percentage

At the first and second assessments after weeks one and two, there was no rooting in any of the five treatments. By week three, treatments 200 and 250 μg displayed significantly higher rooting percentages than 8 μg and 40 μg; which were in turn significantly higher than the control ($P < 0.05$; t-test). Rooting percentage increased at a roughly constant rate, reaching a maximum value at the end of week five (Figure 4.2a), with the highest (66%) percentage rooting registered in the 200 μg treatment.

Plate 4.1: The cuttings of *Irvingia gabonensis* inserted into sawdust to study the effect of auxin (IBA) on their rooting ability.

The control (0 μg), 8 μg and 40 μg treatments did not differ significantly (*P* >0.05; t-test) from each other at the same time. Percentage rooting tended to increase with IBA concentration from week two onwards. Although it was slightly higher in the 200 than in 250 μg by the end of week six, analysis of variance test showed that there was no significant difference (*P* >0.05) between the five treatments (0 μg, 8 μg, 40 μg 200 μg and 250 μg (Table A9).

A deviance stepwise regression analysis revealed that block and node position significantly influenced the rooting ability of the cutting of *I. gabonensis*. But auxin (IBA) was not significantly associated with the cuttings' ability to root (Table A10).

Callus formation

Cuttings did not start to callus until week two, when the percentage of callused cuttings ranged from 92% to 96% in the 0 and 8 μg treatments respectively. Although the proportion of callused cuttings was slightly lower in the control treatment, there were no significant differences in callus formation between the five treatments (*P* >0.05; t-test). From week four to the end of the experiment, all the cuttings irrespective of the treatment had callused (Figure 4.2b).

Cutting mortality

Except for the cuttings that received no auxin treatment, mortality rate remained low in all the hormone treatments until the fourth week, when 2% of the cuttings in the 8 μg treatment died. While this value was maintained in this treatment to week six, mortality rate rose sharply between weeks four and five in treatments 0, 250 and 200 μg (Figure 4.2c), although there were no significant differences (*P* >0.05; t-test) between them. From week five to week six, treatment 250 μg IBA displayed the highest proportion of dead cuttings, while 0% of the cuttings in the 40 μg treatment had died.

Leaf shedding

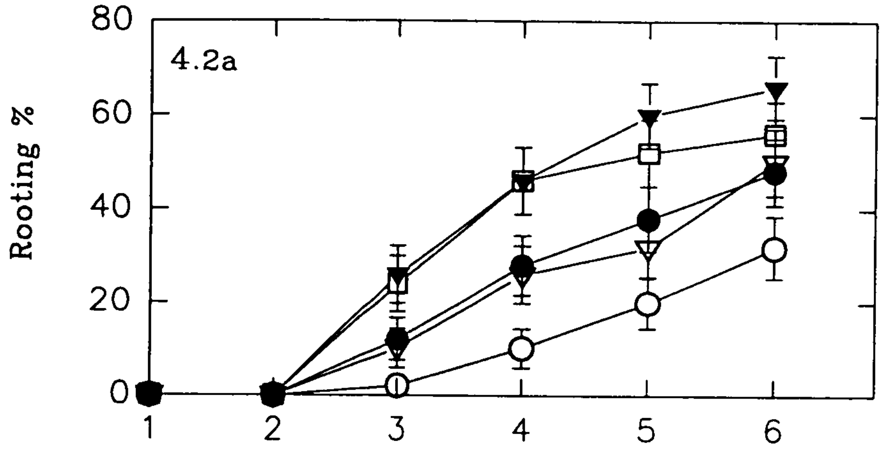
During the first two weeks, leaf abscission occurred in 2% of the cuttings in the 250 μg treatment, rising to 12% by week six. However, there were no significant differences ($P > 0.05$; t-test) between the five treatments in this variable, except treatment 40 μg which was significantly lower ($P < 0.05$; t-test) than treatment 250 μg at the same time. In the 40 μg treatment, leaf loss had commenced at week 5, and by week 6, only 2% of cuttings had lost their leaves.

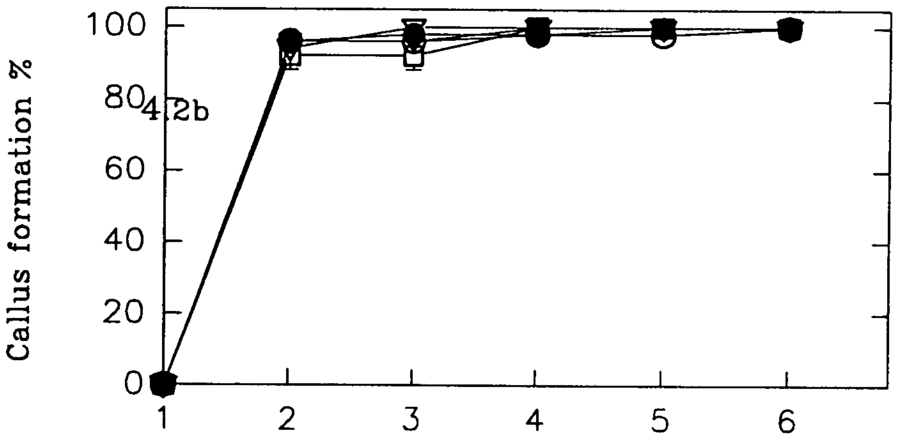
Shoot formation

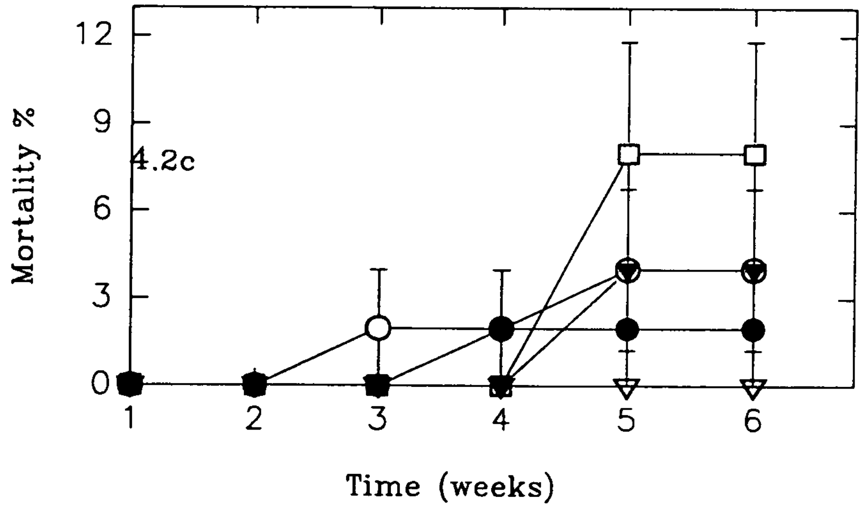
There was no significant difference between the five treatments by the third week, when shoot production commenced. The rate of increase in percentage shoot production was more gradual after week four in each treatment, with a higher proportion of cuttings in the control treatment forming shoots than in the other treatments. However, these differences were not significant ($P > 0.05$; t-test). By the sixth week there was a very narrow range (72-84%) of cuttings sprouting in the five treatments (Figure 4.2e).

Number of roots per rooted cutting

With respect to number of roots per rooted cutting, treatments 200 μg, 250 μg and 8 μg respectively, were significantly higher than treatments 40 μg and 0 μg. Although the 200 μg treatment recorded 4.3 of roots per rooted cutting as against 3.8 and 3.9 in 8 μg and 250 μg treatments, there were no significant differences between these three treatments. Treatments 40 μg and 0 μg recorded the lowest mean root number of 2.3 (Figure 4.2f).


4.2a
Rooting %
0
20
40
60
80
1
2
3
4
5
6


4.2b
Callus formation %
0
20
40
60
80
100
1
2
3
4
5
6


4.2c
Mortality %
0
3
6
9
12
1
2
3
4
5
6
Time (weeks)

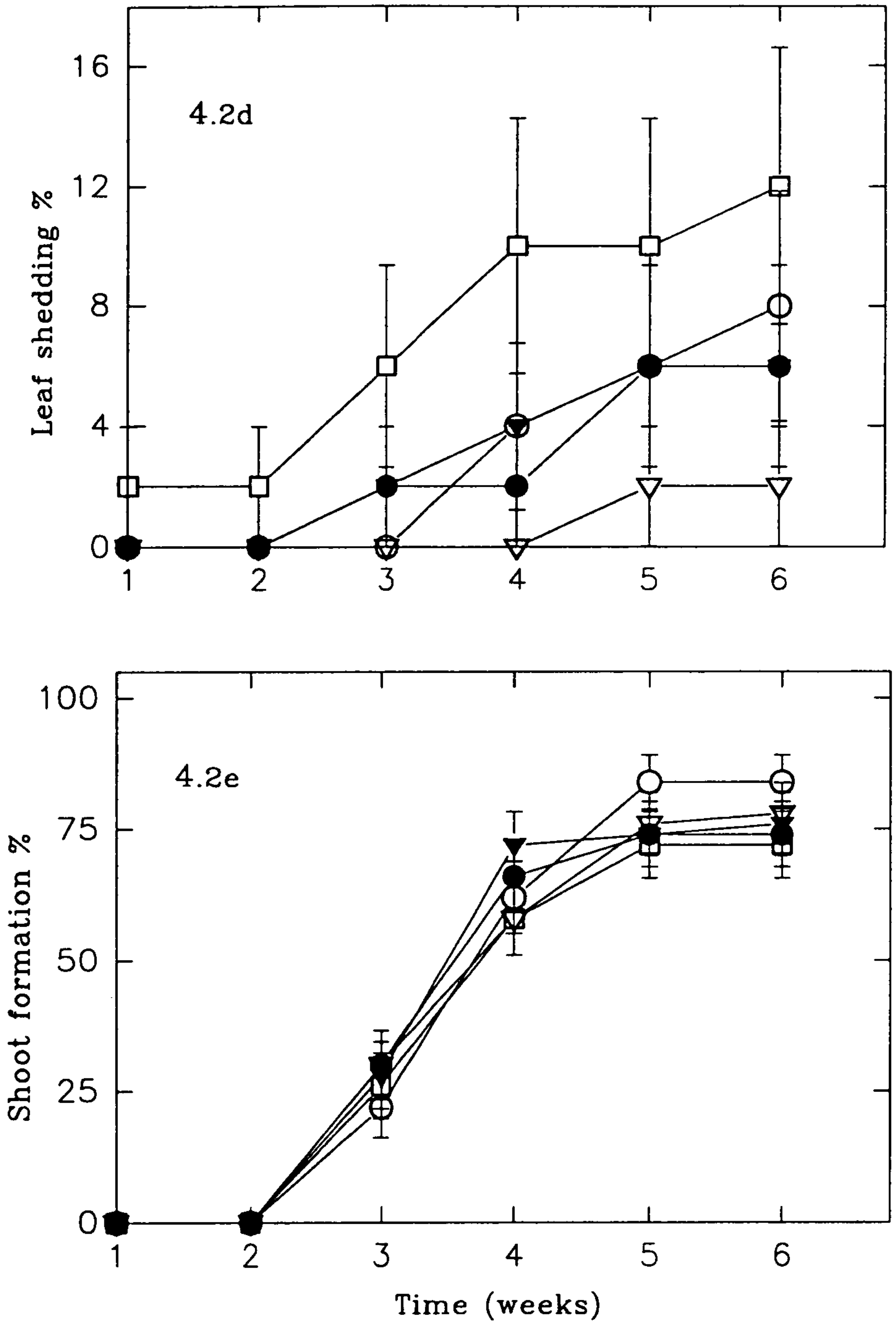


Figure 4.2: Effect of auxin (IBA) concentration on rooting of leafy stem cuttings of *Irvingia gabonensis*, (○ = 0 μg, ● = 8 μg, ▽ = 40 μg, ▼ = 200 μg and □ = 250 μg). Bar = ± SE. (n = 50).

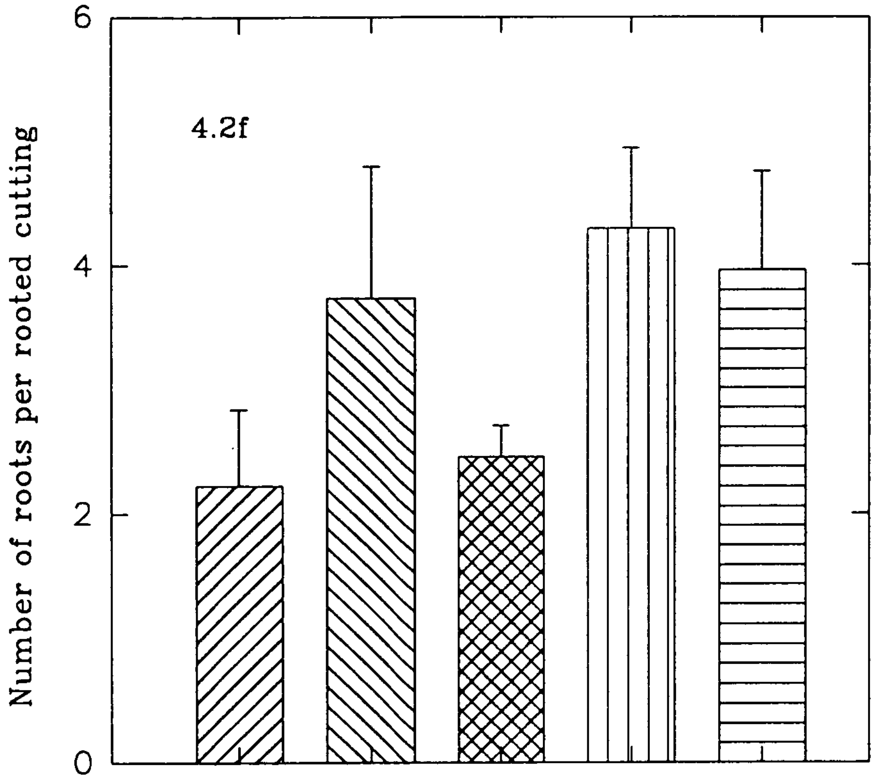


Figure 4.2f: Effect of auxin (IBA) on number of roots per cuttings of *I. gabonensis*., (▨ = 0 μg, ▧ = 8 μg, ▩ = 40 μg, ▥ = 200 μg and ▤ = 250 μg) (after 6 weeks). Bar = + SE. (n = 50).

4.4.0 Experiment 3: Effect of leaf area on rooting of leafy stem cuttings of *Irvingia gabonensis*

4.4.1 Introduction

In many species the leaf area of cutting has a marked influence on rooting, according to experimental conditions (Leakey *et al.*, 1993), although other species display no tendency towards an optimum leaf area (Newton *et al.*, 1992b; Leakey, 1990). No research has been carried out to date to determine the role of leaf retention and area in rooting of *Irvingia gabonensis* cuttings. The present study was, therefore, aimed at investigating the influence of leaf retention and different leaf areas on rooting the cuttings of this species. This is of practical importance as the leaf lamina can be manipulated to enhance rooting for large scale production of clonal stockplants.

4.4.2 Materials and methods

It was intended that the cuttings for this experiment would come from two sources, which were coppice stumps in the bush and young seedling plants sown and raised in the nursery for one year. A visit was made to evaluate coppice shoots of the stumps on the 14th of April 1993. It was discovered that the leaves of the sprout shoots were heavily eaten by defoliating insect larvae. Consequently, cuttings were all obtained from the second source.

On the 15th of April 1993, fifty-five seedling stockplants with orthotropic shoots were selected from which cuttings were taken. Five cuttings were taken from each shoot according to node position. The five treatments were 0 cm^2, 12.5 cm^2, 25 cm^2, 50 cm^2 and 80 cm^2 and were randomly assigned to each of the five cuttings. The single, simple leaves were trimmed to the required area by using a cardboard template cut out of graph paper. After applying 200 μg of indole-3-

butyric acid to the clean-cut base of each cutting (see chapter three) and drying off the alcohol from the base in an air current from a fan, the cuttings were immediately inserted in node order in the sawdust rooting medium. In this way, five shoots provided twenty-five cuttings which were inserted randomly in five lines in the propagator to constitute a block. The fifty-five shoots gave two hundred and seventy-five cuttings in total which were set in eleven such blocks in a randomized manner in the same propagator. Two weeks after insertion, the cuttings were assessed as described in Chapter 3.

Assessment and analysis

After inserting the cuttings into the rooting medium, assessments, analysis of variance and that of deviance were employed to determine treatment effect and that of the factors recorded in this experiment on the rooting ability of the cuttings of this species as described earlier.

4.4.3 Results

Percentage rooting

The results of this experiment showed that leafless cuttings did not produce any roots by the end of the rooting period. While 6% of the cuttings in the 12.5 cm^2 treatment started rooting in the second week, no root production was observed in the other treatments until the third week. By week three, a significantly higher (P <0.05; t-test) proportion (64%) of the cuttings in treatment 80 cm^2 had rooted; the lowest percentage (16%) rooting was recorded in the 12.5 cm^2 treatment. Treatments 25 and 50 cm^2 did not differ significantly (P >0.05; t-test) from each other at this time. By week 4, treatments 50 cm^2 and 80 cm^2 had risen to values of 76% and 87% respectively and were significantly different (P <0.05; t-test) from the other two leafed treatments, although they did not differ from each other.

These differences were maintained until week five, when the 25 cm^2 treatment was significantly higher (*P* <0.001; Table A11) than the 12.5 cm^2 treatment in which the lowest proportion (46%) was registered (Figure 4.1a).

The relative influence of leaf area, block, stem diameter at the cutting base, node position and stem length on the rooting ability of the cuttings of *I. gabonensis* was analyzed by a stepwise regression. The analysis at the end of the experiment showed that rooting was significantly affected by leaf area and node position, but was not significantly affected by block (Table A12).

Callus formation

Cuttings did not produce callus until week three, when there was a significant difference (*P* <0.05; t-test) between four of the five leaf area treatments (0, 12.5, 25 and 40 cm^2). However, there was no significant difference (*P* >0.05; t-test) between the 50 cm^2 and 80 cm^2 treatments, although they were significantly higher than the others. Callusing in all the treatments, except the control, increased until week four. There were no significant differences between three of the four leafed treatments at either the fourth or fifth week (Figure 4.3b). At week four, treatment 12.5 cm^2 was significantly lower than 50 cm^2 and at week five, it was significantly lower than the other three leafed treatments. Percentage callus formation displayed an increasing trend with increasing leaf area in the third week of the experiment, but this tendency disappeared in weeks four and five.

Cutting mortality

By the end of week one after insertion, none of the leafless cuttings had died. The rate of mortality in this control treatment was relatively high, such that by week three, when a percentage mortality of 4% was recorded in the 80 cm^2 leafed treatment, 62% of the leafless cuttings had died. By week five, all of the leafless cuttings were dead. In the leafed treatments, cutting mortality increased very

Plate 4.2: Rooted cuttings of *Irvingia gabonensis* displaying an increasing trend in number of roots per rooted cutting with increasing leaf area.

slightly from week three to five, when a significantly higher (P <0.05; t-test) proportion of 20% of the cuttings in the 12.5 cm^2 treatment had died. The lowest proportion of dead cuttings (4%) was associated with 50 cm^2.

Leaf shedding

Leaf shedding commenced in week two, when 2% of the cuttings in each of the 12.5 and 25 cm^2 treatments had lost their leaves in each case. Percentage leaf shedding increased gradually from week two to week three, when it was significantly higher (P <0.05; t-test) (13%) in the 12.5 cm^2 than it was in the other three leafed treatments, which in turn did not differ significantly (P >0.05; t-test) from each other. The 12.5 cm^2 treatment continued to be significantly higher than the other treatments in this variable from week four to the end of the experiment in week five, when it was associated with 20% leaf shedding. However, there were no significant differences between leafed treatments 25, 50 and 80 cm^2 in this parameter, although the 25 cm^2 treatment was higher than the other two, with 9% leaf loss (Figure 4.3d) at the end of the experiment in week five.

Shoot formation

There was no shoot formation in any leafless cutting throughout the five week period of the experiment. Similarly, none of the cuttings in the leafed treatments formed shoots by week one. But in week two, 2% of the cuttings in the 12,5 cm^2 treatment had produced new shoots, while no shoot had appeared on any of the cuttings in the other three leafed treatments. By week three, percentage shoot formation had increased rapidly to 21.8%, 12%, 11% and 31% in treatments 12.5 cm^2, 25 cm^2, 50 cm^2 and 80 cm^2 respectively. Treatment 80 cm^2 was significantly higher (P <0.05; t-test) than treatments 25 and 50 cm^2 in this variable. However, treatment 80 cm^2 did not differ significantly (P >0.05; t-test) from treatment 12.5

cm^2 in terms of shoot formation by this time. From week three onwards, percentage shoot formation in all the treatments increased at increasing rates to week five, when treatments 12.5 and 80 cm^2 were significantly higher (60% in each of the two treatments) than 25 and 50 cm^2 (35% in each of the two treatments) (Figure 4.3e).

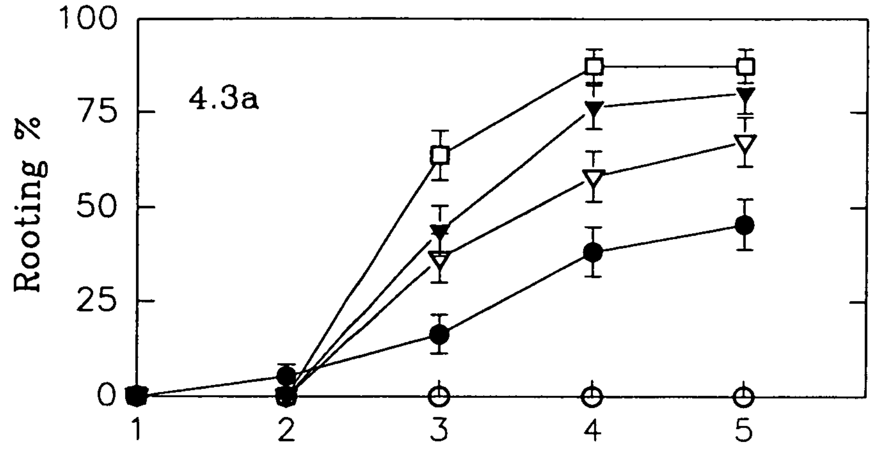
Number of roots per rooted cutting

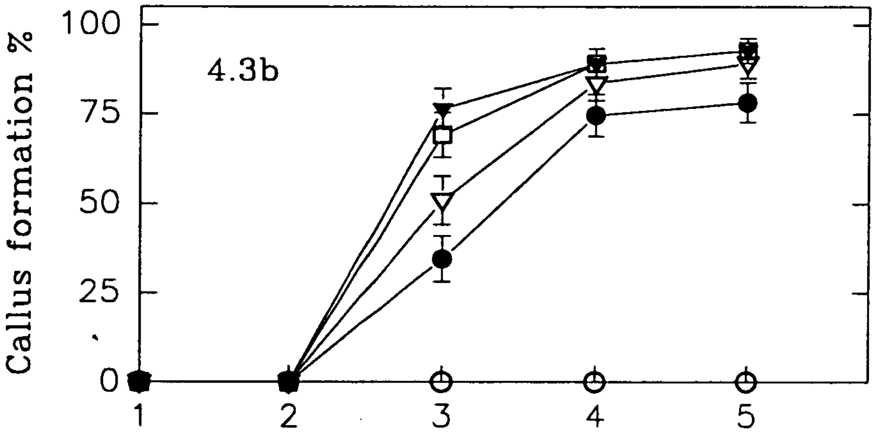
The mean number of roots in this experiment varied very slightly between the four leafed treatments. Mean root number per rooted cutting in the 25 cm^2, 50 cm^2 and 80 cm^2 treatments was significantly higher than in 12.5 cm^2, which was in turn not different from treatments 25 cm^2 and 50 cm^2 after the fifth week of the experiment (Figure 4.3f). Overall mean root number increased with increasing leaf area, with the lowest and the highest numbers (2.6 and 4.1) associated with the 12.5 cm^2 and 80 cm^2 treatments respectively.

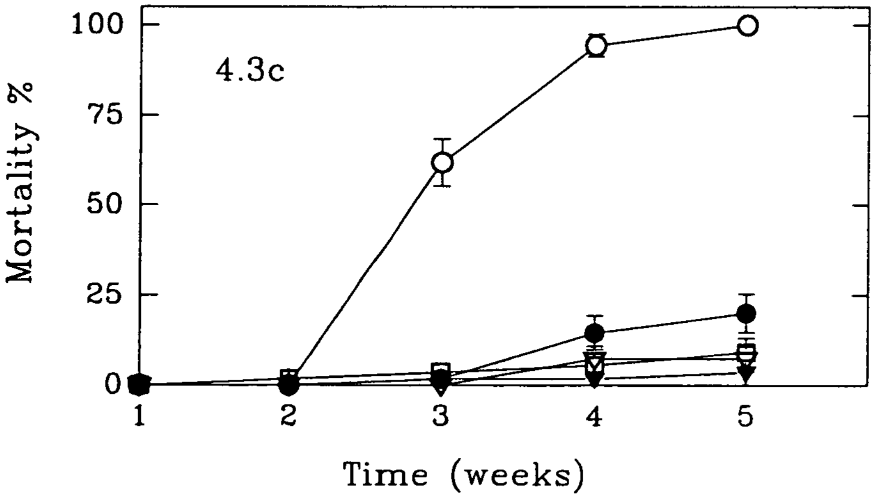
4.5 Discussion

Percentage rooting

It has long been recognised that rooting media, auxin and leaf retention play an important role in enhancing the rooting ability of the cuttings of herbaceous, softwood and hardwood species (Loach, 1985; Morgan and Moustafa, 1989; Breen and Muraoka, 1973; Patrick and Wareing, 1976; Reuveni and Raviv, 1981; Newton *et al.*, 1992b). The highest percentage of rooted cuttings recorded in experiments 1, 2 and 3 were 93%, 66% and 87% respectively. The fact that these values were considerably higher than the controls (32% and 0% in experiments 2 and 3 respectively) confirms the effect of these factors in promoting the rooting ability of the cuttings of this species.


4.3a
Rooting %
100
75
50
25
0
1
2
3
4
5


4.3b
Callus formation %
100
75
50
25
0
1
2
3
4
5


4.3c
Mortality %
100
75
50
25
0
1
2
3
4
5
Time (weeks)

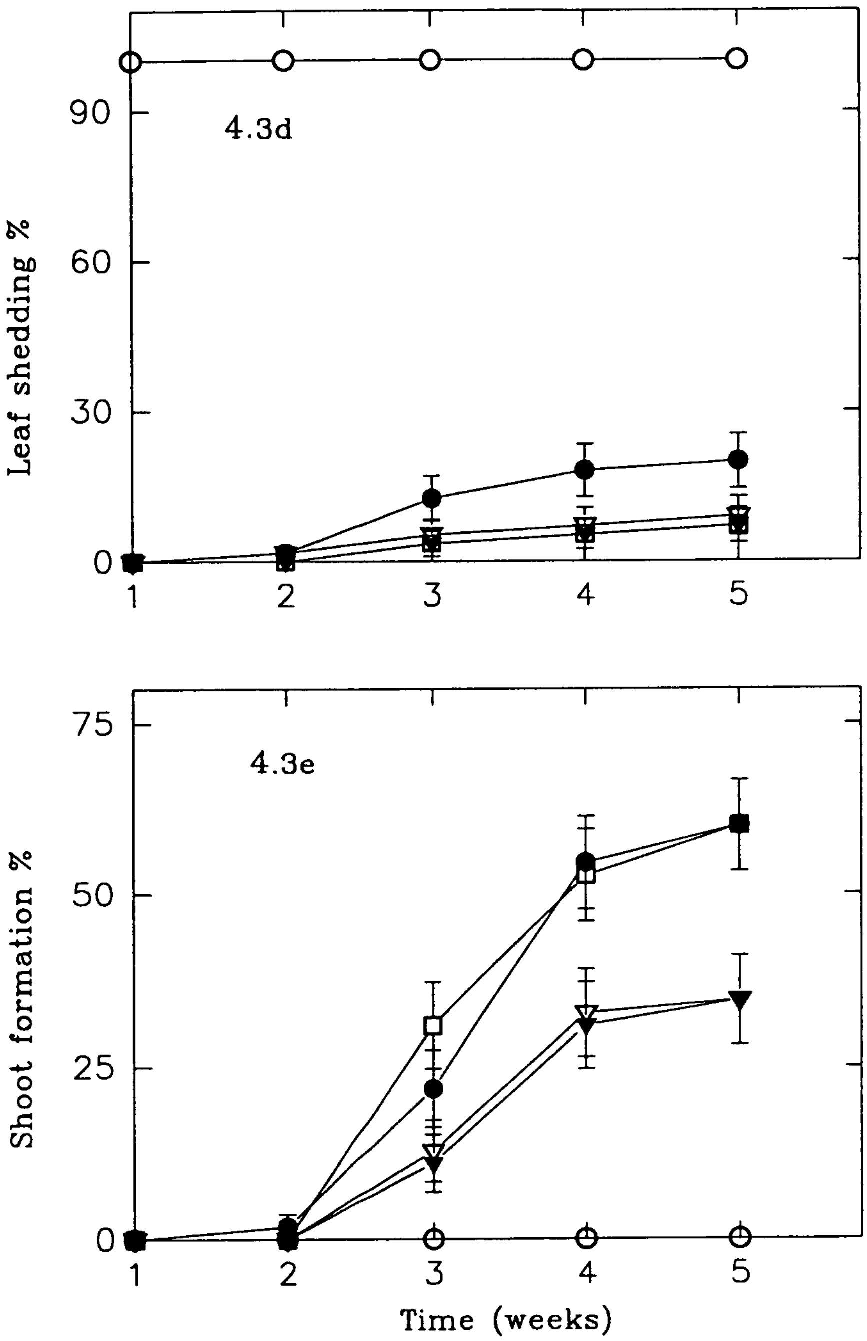


Figure 4.3: Effect of leaf area on rooting of leafy stem cuttings of *Irvingia gabonensis*, (○ = 0 cm^2, ● = 12.5 cm^2, ▽ = 25 cm^2, ▼ = 50 cm^2 and □ = 80 cm^2). Bar ± SE. (n = 55).

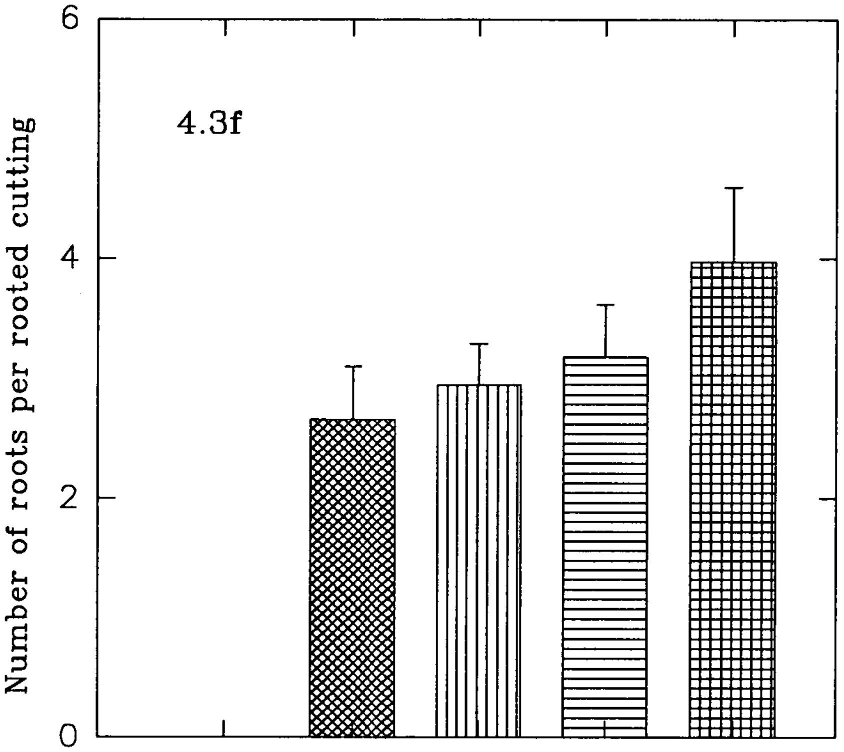


Figure 4.3f: Effect of leaf area on number of roots per cuttings of *I. gabonensis*. ▩ = 12.5 cm^2 ▥ = 25 cm^2 ▤ = 50 cm^2 ▧ = 80 cm^2) (after 5 weeks) (Bar + SE. (n = 55).

In experiment 1, the percentage of rooted cuttings displayed significant differences (*P* <0.001) among the six media tested (Table A7). This indicates that the cuttings of *I. gabonensis* respond differently to the six different media treatments. This agrees with the observation of Hartmann and Kester (1983) that cuttings of some plant species may be greatly influenced by the kind of rooting medium used, both in the percentage cuttings rooted and in the quality of roots produced. Differences in the rooting ability of many tropical tree species in different media were discussed in Chapter 3.

The highest percentage (93%) of rooted cuttings in this experiment was recorded in sawdust. This was closely followed by fine sand (79%) which was not significantly different (*P* <0.05) from it (Table A7). This is consistent with *R. heudelotii* which scored the highest (82%) and second highest (73%) percentage rooting in sawdust and fine sand respectively, although the values are higher in *I. gabonensis* than in the former species. The results in this trait concur with those of *Milicia excelsa* (Ofori, 1994) and those of *Gmelina arborea* (Leakey *et al.*, 1990), but contrast with *Cordia alliodora* (Mesén, 1994), which recorded the lowest (89%) percentage of rooted cuttings in sawdust (see discussion in Chapter 3). This contradicts the rooting results of O'Dell and Stoltz (1978) who recorded a value of 82.3% of rooted cuttings of *Juniperus horizontalis* (Plumosa) in medium sand. These results conform with the observation of Loach, (1985) that the optimum rooting medium differs between species, and that even with related cultivars in the same genus, divergent results can be obtained in different media.

Rooting media have been known to affect rooting for diverse reasons (Loach, 1988; 1985; Andersen, 1986; Hartmann and Kester, 1983). Rooting success or failure has been attributed to the physical characteristics of the rooting media (Park and Andersen, 1989; Andersen, 1986; Loach, 1985; O'Dell and Stoltz, 1978). Optimum volume of gas-filled porespace or porosity, oxygen diffusion rate and water retention capacity are the requirements for an ideal rooting medium

(Hartmann and Kester, 1983; Loach, 1985; Andersen, 1986). Porosity allows adequate aeration which facilitates optimum oxygen concentration at the cutting base (rooting zone) for respiration, creating a basipetal sugar concentration gradient (Dick *et al.*, 1994). This may facilitate callus and root initial formation (Dick *et al.*, 1994). Lower pH values have been shown to inhibit both callus and root production (Hartmann and Kester, 1983). Medium particle size, pathogen infection and microclimate fluctuation may cause rooting variation in cuttings (O'Dell and Stoltz, 1978; Hartmann and Kester, 1983; Howard, 1965). In the present study, the high percentage of rooted cuttings recorded in sawdust and fine sand may be attributed to their relatively high air:water ratios.

In experiment 2, the percentage of rooted cuttings did not display significant differences ($P > 0.05$) between the four IBA treatments and the control (0 μg) (Table A9) in agreement with the results of *R. heudelotii* in Chapter 3. The fact that the untreated cuttings rooted in this experiment indicates that *I. gabonensis* and *R. heudelotii* are similar to *L. trichilioides* (Leakey, 1994) and the other species discussed earlier, which were successfully rooted without auxin application (see discussion in Chapter 3). The lowest value (32%) against the highest value (66%) recorded in the control and in the 200 μg treatment respectively suggests that the application of exogenous auxin more than doubled the rooting ability of this species under the conditions in which this experiment was conducted, as has been demonstrated with many other tropical species (Leakey *et al.*, 1982b; 1990). The percentage of rooted cuttings started to decline at a high dose (250 μg) after it had exhibited an increasing trend with increasing IBA concentration. This illustrates that 200 μg of IBA is the optimum concentration above which this auxin tends to inhibit rooting, perhaps by being toxic to *I. gabonensis* cuttings. These results are in agreement with those of *Cordia alliodora*, *Albizia guachapele* and *Vochysia hondurensis*, where percentage rooting increased with IBA concentration up to an optimum value for each species and then declined with increasing auxin dose (Leakey *et al.*, 1990).

The variation of rooting response with auxin concentration could be attributed to the endogenous auxin status of the cutting at the time of severance (Torrey, 1986; Weigel *et al.* 1984) (see discussion in Chapter 3). The promotive role played by auxin in the rooting of cuttings can be attributed to carbohydrate mobilization, by enhancement of hydrolytic enzyme activity (Nanda *et al.*, 1968b; Rana *et al.*, 1987; Middleton, Jarvis and Booth, 1980) (see discussion in Chapter 3).

The fact that the leafless cuttings of *I. gabonensis* failed to root agrees with *Terminalia spinosa* (Newton *et al.*, 1992b) and *R. heudelotii* cuttings (Chap. 3), although the leafless cuttings of some other species have been shown to root (Hamzah, 1992; Leakey *et al.*, 1982b) (see discussion Chapter 3). This failure to root in the leafless cuttings may be attributed to inability to photosynthesise during propagation (Newton *et al.* 1992b), lack of transpiration and carbon dioxide uptake, and perhaps reduced auxin production (Middleton, Jarvis and Booth, 1980) in the leafless cuttings.

There was a significant difference ($P < 0.001$) between the leafed treatments, with the highest (87%) percentage of rooted cuttings recorded in the 80 cm^2 treatment, while the lowest (46%) was recorded in the 12.5 cm^2 treatment (Table A9). The increasing trend of rooting percentage with increasing leaf area (Figure 4.3a) conforms with the results of *R. heudelotii* in this study, and those of *Eucalyptus camaldulensis* (Geary and Harding, 1984) (Plate 4.2). In addition, the response of the cuttings to increasing leaf area suggests that the optimum leaf area for rooting cuttings of *I. gabonensis* may be higher than those tested in this experiment, in agreement with the observations made on *R. heudelotii* (Chapter 3). Some tropical species have been shown to exhibit optimum leaf areas for maximum root production (Leakey *et al.*, 1982b; Asanga, 1989; Tchoundjeu, 1989), while others have been found to display no tendency towards an optimum leaf area (Newton *et al.*, 1992b ; Leakey, 1990) (see discussion in Chapter 3).

The importance of leaf retention and leaf area in rooting of leafy stem cuttings of most plant species lies in the fact an optimum leaf area strikes a balance between the processes of photosynthesis and transpiration, thereby enhancing rooting (Leakey and Coutts, 1989). Production of current assimilates by the process of photosynthesis, leading the basipetal transportation of carbohydrates to the rooting zone, has been shown to be crucial for adventitious rooting development in a range of species (Reuveni and Raviv, 1981; Newton *et al.*,199b; Dick *et al.*, 1994) (see discussion Chapter 3).

Callusing

The cuttings of experiments 1 and 2 exhibited a similar rate of callusing, all commencing callus production in week two after insertion. In contrast, callus formation did not start in experiment 3 until week three. The percentage of callused cuttings varied slightly among the three experiments in week three, with values of 97%, 93% and 65% recorded in experiments 1, 2 and 3 respectively. At the end of each of these experiments, differences in percentage callusing between treatments were relatively slight.

In experiment 1, callus formation was not affected by any of the six media, while rooting percentage was highly affected. This suggests that callus formation is independent of root production in cuttings. This is in agreement with the observation of Hartmann and Kester (1983) and Van der Lek (cited by Komissarov, 1969), who noted that root formation in cuttings is not connected with the activity of callus. However, it is believed that callus enhances the supply of water to cuttings during propagation in the rooting media (Flerov and Kovalenko, 1952; Buryi, 1901, cited by Komissarov, 1969). But excessive callus formation may inhibit the initiation of adventitious roots (Hartmann and Kester, 1983). However, it has been shown that callus cell masses decrease with increasing alkalinity (pH) of the medium (Hartmann and Kester, 1983) (see discussion in Chapter 3).

By week two, the proportion of cuttings that had callused in experiment 2, ranged from 92% to 96% in the 250 μg and 0 μg treatments respectively. This increased very slightly to the end of the experiment in week six when all the cuttings callused (Figure 4.2b). This indicates that, except in treatment 40 μg, some cuttings callused before they died. Some of them callused but did not root, particularly in the 250 μg and 0 μg treatments. This is consistent with the observation of Newton and Jones (1993a) who found that many cuttings of *Cordia alliodora* produced callus at the base but failed to root. A high proportion (44%) of the cutting which callused in this experiment did not root. This is in contrast to the findings of Uniyal *et al.*, (1993) who reported that although callus formation was seen on cuttings of all treatments, further differentiation into roots was achieved only in auxin treated cuttings of *Dalbergia sericea*. Lack of sufficient nutrient reserves, internal factors and the cutting age have been implicated in the non-differentiation of callus at the cutting base into roots (Bonga and Durzan, 1982). This might have been the case in this experiment.

The leafless cuttings of *I. gabonensis* failed to callus in this experiment. In this connection, this species is similar to *R. heudelotii* where the leafless cuttings failed to callus in leaf area experiment in the present study. However, the cuttings in the leafed treatments callused, such that by week three after insertion, the percentage of callused cuttings ranged from 35% to 76% in the 12.5 cm^2 and 50 cm^2 respectively, with many more cuttings callusing in the smaller than in the larger leaf area treatments. This pattern was maintained to the end of the experiment in week five, when the percentage of callused cuttings displayed an increasing trend with increasing leaf area. This is consistent with the percentage of rooted cuttings, which increased with increasing leaf area. In view of this, *I.*

gabonensis is likened to *R. heudelotii* where the cuttings exhibited the same pattern of behaviour in these two variables.

The fact that both the percentages of rooted and callused cuttings increased with increasing leaf area suggest that callus and root formation are closely linked together in this experiment. It would appear, therefore, that in some cases rooting is dependent on callus formation. This is contrary to the views of Hartmann and Kester (1983) and Van der Lek (1925, cited by Komissarov, 1969) that callus formation in cuttings of most species is independent of rooting. The lack of sufficient nutrient reserves (as a result of little current assimilate from smaller leaf areas) at the base of the cuttings with smaller leaf area may account for the failure to develop further into roots (Bonga and Durzan, 1982).

Mortality

Cutting mortality varied considerably among the three experiments. Mortality started in the third week in experiment 2, while it did not commence in experiments 1 and 3 until week four. The rate of mortality rose sharply in experiments 1 and 2 to values of 13% and 8% respectively, while in experiment 3, a highest value of 20% was recorded at the end of the experiment.

In experiment 1, while the highest cutting mortality of *I. gabonensis* was recorded in gravel, that of *R. heudelotii* was associated with the mixture of gravel and sawdust. *I. gabonensis* produced the lowest cutting mortality (0%) in sawdust and the mixture of medium sand and sawdust, while lowest mortality of *R. heudelotii* was recorded in sawdust. These results are similar to those of Ofori (1994) who recorded the lowest cutting mortality of *Milicia excelsa* in sawdust, but contrary to those of Mesén (1994) who observed the highest cutting mortality of *Cordia alliodora* in the same rooting medium.

While the cuttings of *R. heudelotii* died mostly by rotting, most of the cuttings of *I. gabonensis* died by desiccation. In some cases, the cuttings shed their leaves before dying and in other instances the basal end died while the top and the leaves were still alive. Rooting was even observed in some cuttings which were partially dead. In the case of rotting at the base, cutting mortality could be attributed to waterlogging of the rooting medium (O'Dell and Stoltz, 1978), particularly after leaf abscission, when transpiration was arrested (see discussion in Chapter 3).

The general causes of cutting mortality in rooting media are attributed to the inappropriate proportions of the physical properties of the media such as air, water, porosity and solid particle size (O'Dell and Stoltz, 1978; Loach, 1985; 1988), and infection of mycotic or bacterial origin (Wells, 1955; Hartmann and Kester, 1983) (see discussion in Chapter 3).

In experiment 2, the highest mortality was recorded in the highest auxin concentration. This suggests that high doses of IBA may be toxic to *I. gabonensis*, as has been observed in other species (Middleton *et al.*, 1978). The likely optimum concentration of IBA for rooting the cuttings of *I. gabonensis* is 40 μg. However, the percentage of cutting mortality was much higher (40%) in the highest IBA treatment in *R. heudelotii* than it was in that of *I. gabonensis*. This further demonstrates that the high auxin toxicity in *R.heudelotii*, and low auxin requirements for rooting as compared to those in *I. gabonensis*, which may be attributed to the relatively soft and hard stem tissues of the former and the latter species respectively. This conforms with the view that hardwood cuttings require higher auxin concentrations than softwood cuttings for maximum root production (Hartmann and Kester, 1983; Loach, 1985; 1988). Cutting mortality associated with high auxin concentrations may be attributed to the toxicity of these growth regulators to the cutting tissues (Nanda *et al.*, 1968a; Rana *et al.*, 1987; Middleton, Jarvis and Booth, 1980; Haissig, 1986;) (see discussion in Chapter 3).

Cutting mortality in experiment 3 tended to decrease with increasing leaf area. However, lowest mortality was recorded in the 50 cm^2 leaf area treatment, whereas the highest percentages (87%) of rooted and (92.7%) of callused cuttings were recorded in the 80 cm^2 treatment. The low cutting mortality in the 50 cm^2 leaf area treatment could be ascribed to the fact that at this leaf area, an optimum balance was obtained between transpiration and photosynthesis (Leakey and Coutts, 1989) for the survival of cuttings of this species. The smaller leaf areas may have produced inadequate amounts of carbohydrate for cutting survival.

Leaf abscission

The variation in percentage leaf shedding in all the three experiments was more pronounced from the third week onwards to the end of each experiment, with the highest values of 46%, 12% and 20% associated with experiments 1, 2 and 3 respectively. By the end of experiment 1 at week five, the percentage of cuttings that had lost their leaves was significantly higher (46%) in gravel than it was in the other media. On the other hand, the lowest proportion of cuttings that lost leaves was recorded in sawdust and fine sand. Interestingly, these are the treatments in which the first and second highest percentage rooting and callusing were recorded respectively. In this regard, leaf abscission was found to be negatively related to percentage rooting and callus formation. In terms of leaf abscission, *I. gabonensis* is similar to *R. heudelotii*, which shed the lowest percentage of leaves in the sawdust medium. Similarly, leaf shedding percentage in the cuttings of both species was equally low in the fine sand medium.

Leaf abscission has been ascribed to water stress which could result from either the low moisture content of the medium (Gislerod, 1983), or waterlogging, which could cause anoxia leading to leaf shedding (Loach, 1985). Leaf shedding in cuttings may also be attributed to their position of on the shoot and to the age of the shoot from where they are taken (Leakey *et al.*, 1994). Basal nodes which are more lignified tend to be associated with higher concentrations of abscisic acid which causes leaf abscission in cuttings (Hartmann and Kester, 1983). All or most of these factors might have been responsible for leaf abscission in this experiment.

In experiment 2, at the end of the experiment, the percentage leaf abscission ranged from 2% to 12% in the 40 μg and 250 μg treatments respectively, while 6%, 6% and 8% leaf loss were associated with treatments 200 μg, 8 μg and 0 μg (control) respectively. It is interesting to observe that the lowest proportion (0%) of cuttings that died were recorded this treatment, which scored the lowest percentage leaf abscission. This is in contrast with *R. heudelotii* where the highest proportion of cuttings that shed their leaves was registered in the 40 μg treatment, but the highest percentage rooting and callusing were recorded in the same treatment. The fact that the highest proportion of leaf abscission was registered in the highest auxin concentration suggests that this dose was above the optimum for *I. gabonensis*. Consequently, it might have become toxic to the cuttings resulting in leaf shedding.

From week two to the end of experiment 3 at week five, percentage leaf shedding increased progressively to 7%, 7%, 9% and 20% in treatments 80 cm^2, 50 cm^2, 25 cm^2 and 12.5 cm^2 respectively. Leaf abscission tended to increase with decreasing leaf area. These results agree with those of *R. heudelotii*, where percentage leaf loss was negatively related to leaf area, but contradict those of *Terminalia spinosa* (Newton *et al.*, 1992b) and those of *Khaya ivorensis* (Asanga, 1989), where larger leaf areas tended to abscise more than smaller leaf areas (see discussion in Chapter 3). Higher percentage of leaf shedding in the smaller leaf area could be ascribed to the inability of the smaller leaf areas to maintain a positive carbon balance (see earlier discussion). The highest leaf shedding was found to be associated with the 12.5 cm^2 leaf area treatment in which the highest cutting mortality was recorded.

Shoot formation

The rate of shoot formation in the three experiments was similar in week one. However, by week two, three times more cuttings had formed shoots in experiment 1 than in experiments 2 and 3. Percentage shoot formation increased at different rates in each of the three experiments. Percentage shoot formation ranged from 33% to 76%, 72% to 84% and 35% to 60% in experiments 1, 2 and 3 respectively at the end of each experiment. This indicates that the highest shoot percentages were produced in the auxin experiment.

In the media experiment, percentage shoot formation started in the second week after cutting insertion and increased progressively to the end of the experiment in week five, when there were significant differences between the six media in this trait. The highest proportion of cuttings that formed new shoots was recorded in fine sand (76%) and this was closely followed by sawdust (71%). Sawdust and fine sand equally enhanced the rooting and callusing of the cuttings of this species. In this regard, *I. gabonensis* is similar to *R. heudelotii*, where the cuttings formed the highest percentage shoots in sawdust. This demonstrates that shoot formation and callus production in these two species are both positively related to percentage rooting, which are considerably enhanced by sawdust and fine sand.

In experiment 2, cuttings in all treatments did not start to produce shoots until week three when new shoot formation ranged from 22% to 30% in the control and 40 μg treatments respectively. From week three to the end of the experiment in week six, there were no significant differences ($P > 0.05$; t-test) between the treatments in this variable. However, the highest percentage (84%) of shoot formation was recorded in the control treatment (0 μg). The highest callusing percentage of cuttings was also found in this treatment. However, the lowest percentage of rooted cuttings was recorded in this treatment. This demonstrates that callus and shoot formation do not relate to the rooting ability of the cuttings of *I. gabonensis* except when an exogenous auxin has been applied. The results agree with those of Uniyal *et al.* (1993) who found that new shoots appeared on all cuttings of *Dalbergia sericea* irrespective of auxin concentration treatment (see discussion in Chapter 3).

New shoot formation in cuttings during propagation has been attributed to the availability of carbohydrate reserves (Wright, 1975), current production of assimilates (Newton *et al.*, 1992b) and leaf trimming (Geary and Harding, 1984) (see discussion in Chapter 3).

Although only 2% of the cuttings in the 12.5 cm^2 leaf area treatment had produced new shoots by week two, there were significant differences (P <0.05; t-test) between the four leaved treatments in this variable by week five, when 60%, 35%, 35% and 60% of the cuttings produced shoots in treatments 12.5 cm^2, 25 cm^2, 50 cm^2 and 80 cm^2 respectively. All the leafless cuttings had died before this time. This contradicts the observation on leafless cuttings of *Terminalia spinosa*, which sprouted and produced new leaves (Newton *et al.*, 1992b). The fact that the highly trimmed leaved cuttings responded by sprouting agrees with Geary and Harding (1984), who stated that trimming stimulates foliar bud development in cuttings during propagation.

Root number

The mean root number per rooted cutting ranged from 3.2 to 7.8, 2.2 to 4.3 and 0 to 4.0 in experiments 1, 2 and 3 respectively. The zero value was recorded in the leafless cuttings in experiment 3. The highest mean root number per rooted cutting was recorded in sawdust in experiment 1, in the 200 μg treatment in experiment 2 and in the 80 cm^2 leaf area treatment in experiment 3. This is consistent with the observations on rooting percentage.

In the media experiment, the highest mean root number per rooted cutting was recorded in sawdust. This observation agrees with *R. heudelotii* and with other tropical species, such as *Milicia excelsa*, where the highest mean root number per rooted cutting and the highest percentage of rooted cuttings were recorded in sawdust (Ofori, 1994). It is also interesting to note that the lowest mean number of roots per rooted cutting for all three species was registered in gravel. The high number of roots per rooted cutting in this experiment may be attributed to the high air:water ratio and the other physical properties of the rooting media (see earlier discussion).

In experiment 2, the mean number of roots per rooted cutting varied considerably between the five treatments. However, the highest value in this experiment was recorded in the 200 μg treatment in which the highest percentages of rooted and callused cuttings were also recorded.

The mean number of roots per rooted cutting in experiment 3 increased with increasing leaf area such that the highest value of 4.0 was recorded in the highest leaf area treatment of 80 cm^2. In this respect, *I. gabonensis* is similar to *R. heudelotii*, where the mean number of roots per rooted cutting was positively related to the leaf area. This is consistent with Breen and Muraoka (1974) who found that the mean number of roots per rooted cutting of *Prunus cerasifera* and *Prunus munsoniana* increased with increasing leaf number. The highest percentages of rooted and callused cuttings in this experiment were also recorded in the 80 cm^2 leaf area treatment. It would appear that the leaf area above which the percentage of rooted cuttings, callused cuttings and mean number of roots per rooted cutting may start to decline is greater than 80 cm^2.

CHAPTER 5

Effects of different rooting media, IBA concentrations and leaf areas on rooting of leafy stem cuttings of *Gnetum africanum*

CHAPTER 5

5.0 Effects of different rooting media, IBA concentrations and leaf areas on rooting of leafy stem cuttings of *Gnetum africanum*

5.1.0 Introduction

The leaves of *Gnetum africanum* are valued as a green vegetable and for medicinal preparation (see chapter one). Various morphological (Mialoundama, 1979; 1980; Mialoundama and Paulet, 1985), physiological (Mialoundama, 1990; Abani,1988), nutritional (Fokou and Domngang, 1989; Bahuchet, 1990) and biochemical (Ouabonzi *et al.*, 1983) studies have been carried out on this climber, with a view to understanding its physiological functioning and its ecological characteristics. Little research has been undertaken on the silviculture of this species, although some successful attempts to propagate it by cuttings in Nigeria were reported by Okafor (1980).

In this chapter, experiments aimed at studying the effects of three factors affecting rooting were carried out on leafy stem cuttings of *Gnetum africanum.* The experiments examined how different rooting media, auxin (IBA) concentrations and leaf areas influenced rooting ability.

5.2.0 Experiment 1: Effect of rooting media on rooting of leafy stem cuttings of *Gnetum africanum*

5.2.1 Introduction

This experiment consisted of six rooting media treatments, prepared as described in chapters two and three, namely: SD, FS, G:SD, MS, G and MS:SD. The aim was to evaluate the rooting performance of cuttings of this vine in the six media, and to subsequently select the appropriate rooting medium for mass propagation.

5.2.2 Materials and methods

In the first week of February 1993, nine of the *G. africanum* collection sites were visited with some of the women who harvest this vegetable in the Southern Bakundu Forest Reserve. After this visit, four locations were selected on the basis of large leaf area, high yield and accessibility; and numbered as locations 1 to 4. On the 24th of February 1993, twelve vines were collected from each of the four locations. Seven cuttings were taken from each vine. A total of eighty-four cuttings were harvested from each location. After trimming the two opposite leaves at the node to about 50 cm^2 and applying 200 μg (IBA) to the clean-cut base of each cutting, fourteen cuttings from each location were inserted according to node position in each of the six media (Plate 5.1 A & B). After one week, the cuttings were assessed as described earlier.

Assessment

Assessments of factors affecting rooting were carried out as described in earlier experiments. Six compartments each measuring one meter by one meter were placed in the same propagator. Six different rooting media were put in them. Analysis of variance and that of deviance for stepwise regression were used to assess the effect of the media and the other factor recorded in this experiment on the rooting ability of the cuttings of *G. africanum* as in earlier experiments.

5.2.3 Results

Rooting percentage

Rooting did not start until the third week when the percentage of rooted cuttings

Plate 5.1 A & B: Cuttings of *Gnetum africanum* inserted into sawdust to study the effect of media (A) and leaf area (B) on their rooting ability.

did not differ significantly ($P > 0.05$; t-test) between the six rooting media. Rooting percentage then increased rapidly to week four when it was significantly lower ($P < 0.05$; t-test) in G:SD than in the other media and significantly higher in SD and MS than in the rest. The percentage of rooted cuttings then rose more gradually to week five, when it was significantly higher ($P < 0.05$; t-test) in SD than it was in the other five media. At the same time, the proportion of rooted cuttings was higher in FS than it was in the other four media within which there were no significant differences (Figure 5.1a). This trend was maintained from the fifth to the eighth week of the experiment, when analysis of variance test showed that there were significant differences ($P < 0.05$) among the six media tested (Table A13), with a range of 43% to 82% rooted cuttings recorded in G:SD and SD respectively.

When the results were analyzed by stepwise regression, cutting stem diameter at the base media were found to affect the rooting ability of *G. africanum* significantly, but block did not significantly affect the ability of the cuttings to root (Table A14).

Callus formation

Although no callus formation was observed in the first two weeks after insertion, callusing was recorded in SD (5%) and FS (2%) by the third week. By week four the percentage of callused cuttings had increased sharply, with values in SD, G and MS significantly higher than those in the other media, but not significantly different from each other. MS:SD and G:SD were significantly lower than the other media in this variable and differed significantly ($P < 0.05$; t-test) from each other, with MS:SD having a higher percentage (32%) than G:SD (16%). From week five to eight, percentage of callused cuttings remained significantly higher in SD than in the other media reaching a maximum of 86% at week eight. In contrast, the percentage callusing in the G:SD medium remained relatively low

(16% from week four to 48% in week eight) throughout the experiment (Figure 5.1b).

Cutting mortality

No cutting died in any of the six media by the second week of this experiment, but by week three 7% of the cuttings had died MS:SD, while in FS 2% of the cuttings had died. With the exception of SD, the rate of cutting mortality increased in all treatments progressively until the sixth week, when percentage cutting mortality was significantly higher (46%) in MS:SD than in the other media. This was still the case by week seven, when in addition, mortality in SD was significantly lower (13%) than in the other treatments. The other media did not differ significantly from each other ($P > 0.05$; t-test). These differences were maintained until the eighth week when the experiment was concluded.

Leaf shedding

In this experiment, none of the cuttings in any of the six media treatments shed leaves in the first three weeks, but by week four, leaf shedding had commenced in all media except SD. Leaf shedding percentage increased rapidly between weeks three and six except in SD. At the eighth week, the proportion of cuttings that had shed their leaves was significantly ($P < 0.05$; t-test) lower in SD (13%) than in the other media. However, the percentage of leaf loss was significantly higher ($P < 0.05$; t-test) in MS:SD than in the other four media within which there were no significant differences ($P > 0.05$; t-test). The highest value of 48% was recorded in MS:SD, although the five media other than SD did not differ significantly from each other (Figure 5.1d).

Shoot formation

In this experiment, no cutting in any of the six media produced a new shoot when the experiment was in progress. After potting and nursing for a week or two, however, most of them started sprouting.

Number of roots per rooted cutting

The mean number of roots per rooted cutting after eight weeks was significantly higher in the FS, G, MS and SD media than in the MS:SD and G:SD treatments, although these four media did not differ significantly (*P* >0.05; t-test) from each other. The number of roots in G:SD and MS:SD was significantly lower than in the other four media, but not significantly different from each other (*P* <0.05; t-test).

5.3 Experiment 2: Effect of different IBA concentrations on rooting of leafy stem cuttings of *Gnetum africanum*

5.3.1 Introduction

In this experiment, the effect of five concentrations of auxin (IBA) on rooting of leafy stem cuttings of *Gnetum africanum* was tested, with the objective of selecting the appropriate dose for optimum rooting.

5.3.2 Materials and methods

Fifty-five vines of *Gnetum africanum* were collected from the Southern Bakundu Forest Reserve. They were taken in an icebox to the propagation unit where five cuttings were obtained from each of them.

After trimming the leaves of each cutting to about 50 cm^2, five auxin treatments were randomly applied to each of the five cuttings from each shoot. The cuttings were then inserted, according to node position, in each of five lines within the propagator to constitute a block of twenty-five cuttings (5 treatments per vine of 5 cuttings x 5 vines). The treatments were 0 μg, 8 μg, 40 μg, 200 μg and 250 μg IBA. These doses were prepared by weighing and dissolving 0.008 g, 0.04 g,0.2 g and 0.25 g respectively of indole-3-butyric acid powder in 10ml of industrial alcohol. A dose was applied to the clean-cut base of each cutting by a 10 μg drop from a microsyringe. The control was a drop of 10 μl of industrial alcohol. All five vines were treated in the same manner and cuttings from them were inserted to complete block one. This was replicated eleven times to give eleven blocks. The rooting medium used in this experiment was sawdust.

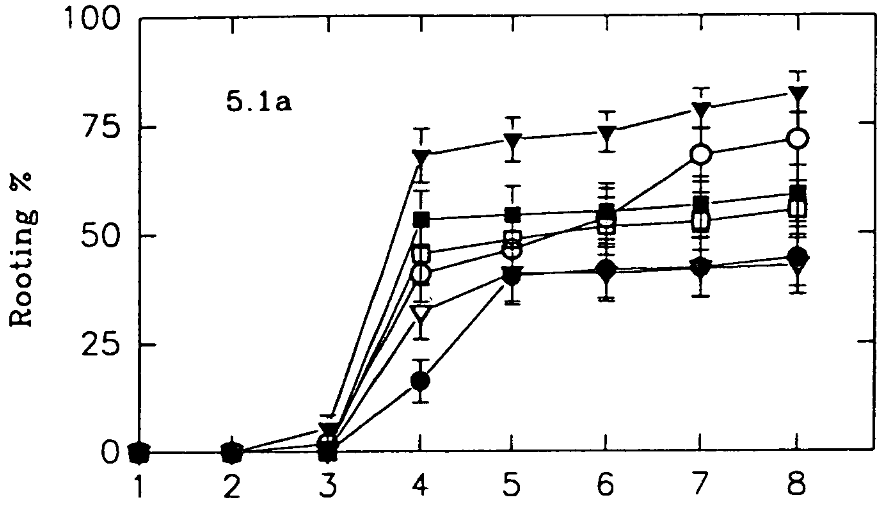
Assessment and analysis

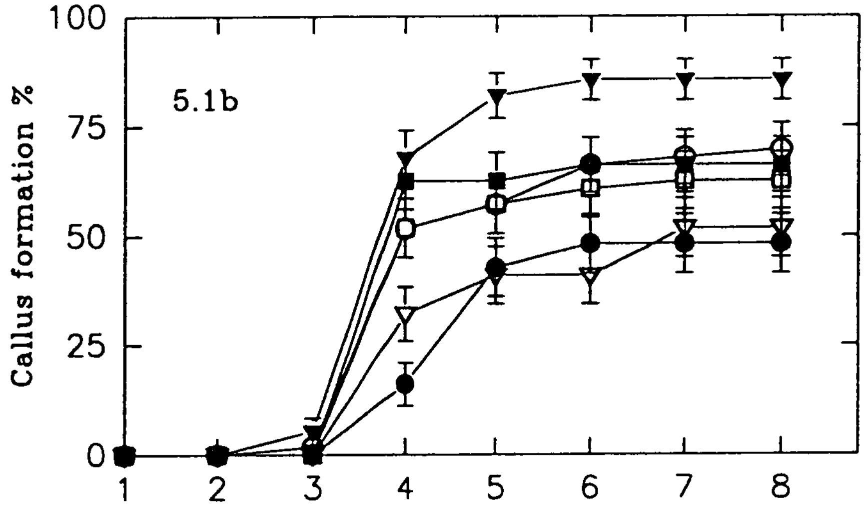
One week after insertion, cuttings were assessed on a weekly basis for root formation, leaf loss, new shoot formation, callus production and changes in cutting and root diameters and at the end of the experiment in week eight, analysis of variance and that of deviance were employed to study the effect of different leaf areas and the other factors investigated in this experiment on the rooting ability of *G. africanum.*

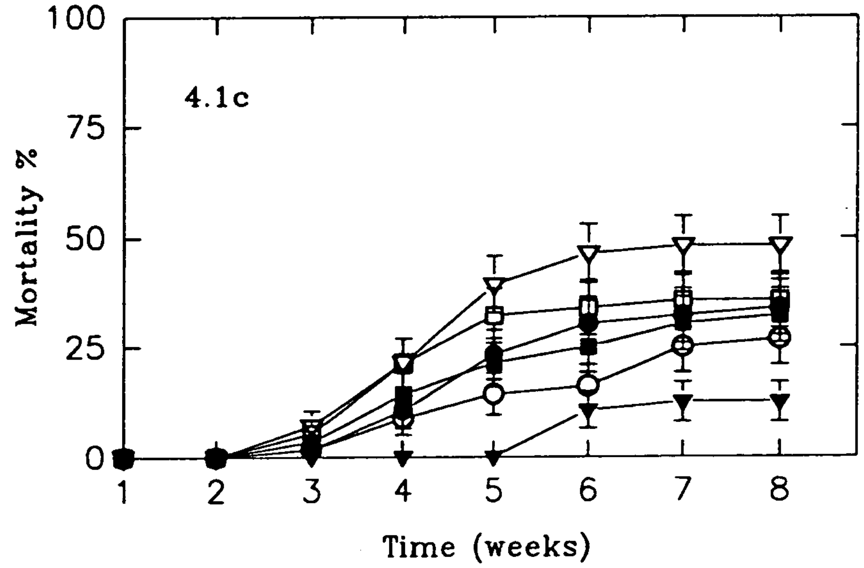
5.3.3 Results

Rooting percentage

Rooting did not start until week three when rooting percentages tended to increase with increasing auxin (IBA) dose (Figure 5.2a). By week five, percentage rooting ranged from 51% to 71% in the 0 μg and 250 μg treatments respectively. The 0 μg treatment did not differ significantly from the other treatments (8 μg, 40 μg,


5.1a
Rooting %
0
25
50
75
100
1
2
3
4
5
6
7
8


5.1b
Callus formation %
0
25
50
75
100
1
2
3
4
5
6
7
8


4.1c
Mortality %
0
25
50
75
100
1
2
3
4
5
6
7
8
Time (weeks)

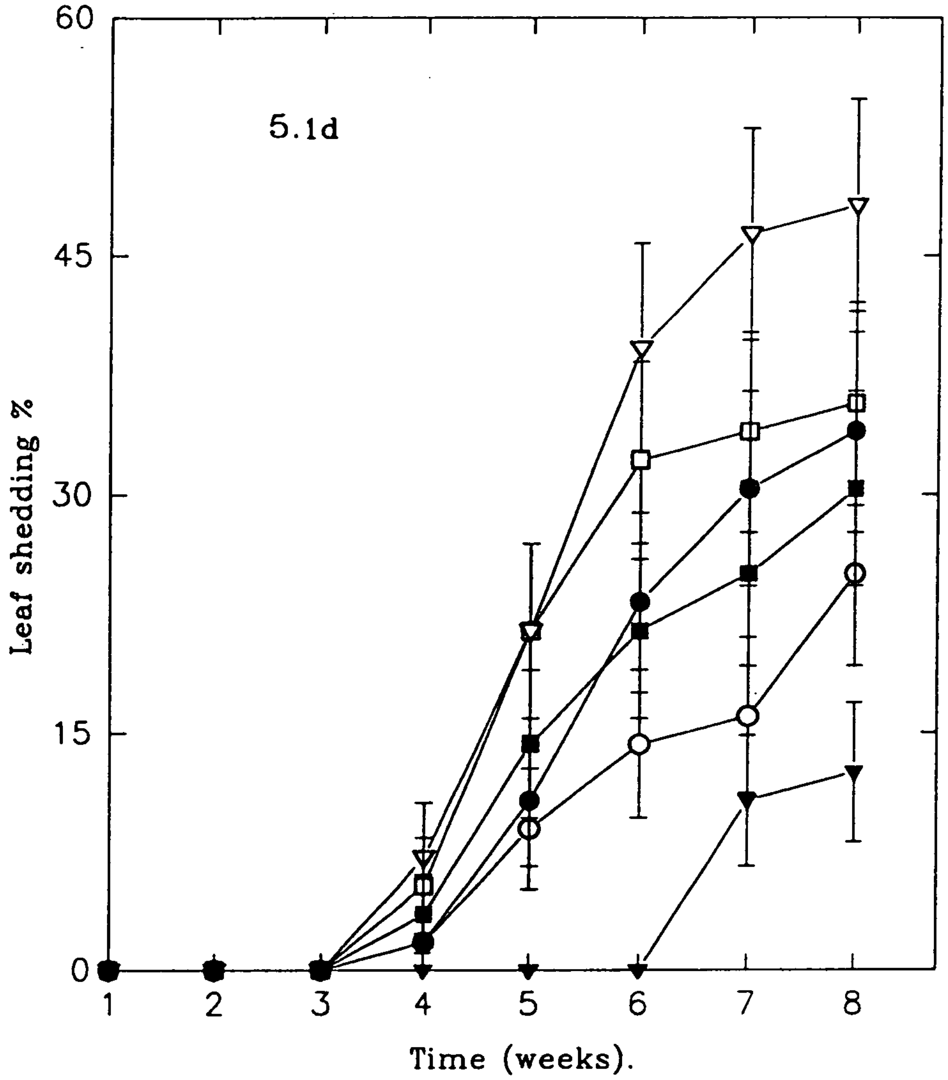


Figure 5.1: Effect of different rooting media on rooting of leafy stem cuttings of *G. africanum*, (▼ = SD, ○ = FS, ■ = MS, □ = G, ▽ = MS:SD and ● = G:SD). Bar ± SE). (n = 56).

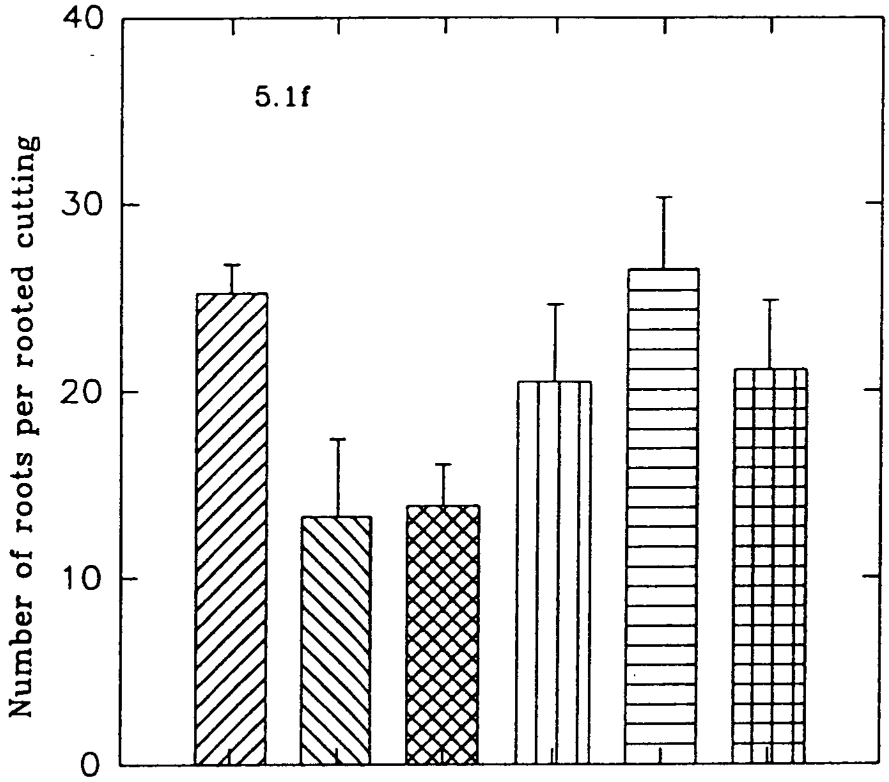


Figure 5.1f: Effect of different media on number of roots per cuttings of *G. africanum*, ▨ = SD, ▤ = FS, ▦ = MS, ▥ = G, ▩ =,MS:SD and ▧ = G:SD). Bar = + SE.. (6 weeks after insertion). (n = 56).

200 μg and 250 μg (ANOVA; P >0.05; Table A 15). Rooting percentages ranged from 51% to 71% in 0 μg and 250 μg treatments respectively by week five (Figure 5.1a).

A deviance stepwise regression analysis showed that block, stem diameter at the cutting base, stem length and auxin concentration did not have significant effect on the rooting ability of the cuttings of *Gnetum africanum* (Table A16).

Callus formation

By the end of week one, 2% of the cuttings in the 200 μg treatment had callused. In the second week, callus formation had taken place in all the treatments, with percentage of callused cuttings ranging from 46% to 62%, in treatments 8 and 250 μg respectively. Callus formation increased between weeks three and five when the 8 μg treatment reached a value of 98% of the cuttings having callused, while the 250 μg treatment displayed the lowest value of 80%. There were no significant differences (P >0.05; t-test) between the treatments in terms of percentage callus formation at any time.

Cutting mortality

By week one, 2% of the cuttings treated with 250 μg of IBA had died. This increased to 13% in week two, when treatments 0, 8 and 40 μg had lost the lowest proportion of cuttings (2%, 2% and 0% respectively). While cutting mortality remained at 2% throughout the five weeks of this experiment in treatment 8 μg, percentage mortality tended to increase in the other treatments with time (Figure 5.2c). There were significant differences between two treatment pairs, namely treatments 8 and 0 μg, and 200 and 250 μg (P <0.05; t-test) but there were no significant differences between treatments within each pair throughout the experiment.

Leaf shedding

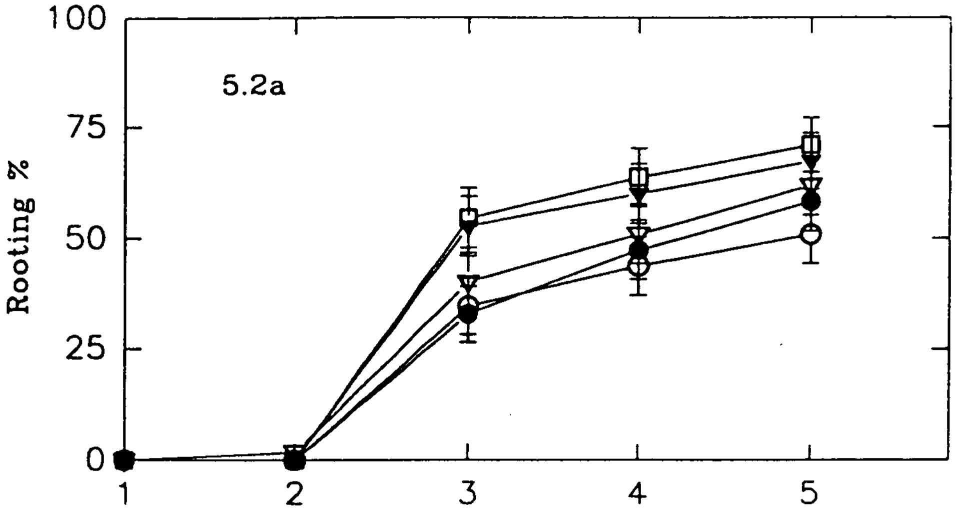
Leaf shedding commenced in the first week of the experiment, with treatment 250 μg recording the highest percentage, and treatments 0 and 8 μg scoring the lowest. The proportion of leaves shed was significantly higher in treatment 250 μg, and significantly lower in treatment 8 μg than it was in treatments 200, 40 and 0 μg ($P < 0.05$; t-test), at the end of week five (Figure 5.2d).

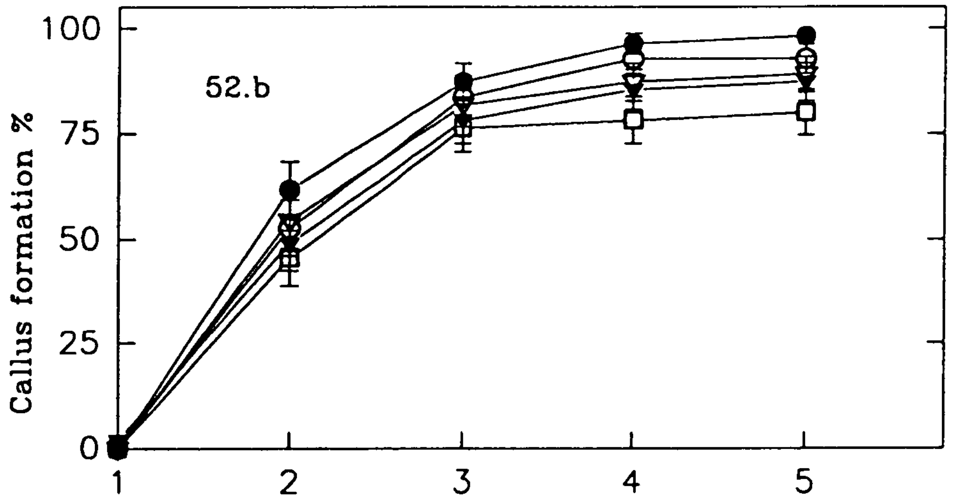
Shoot formation

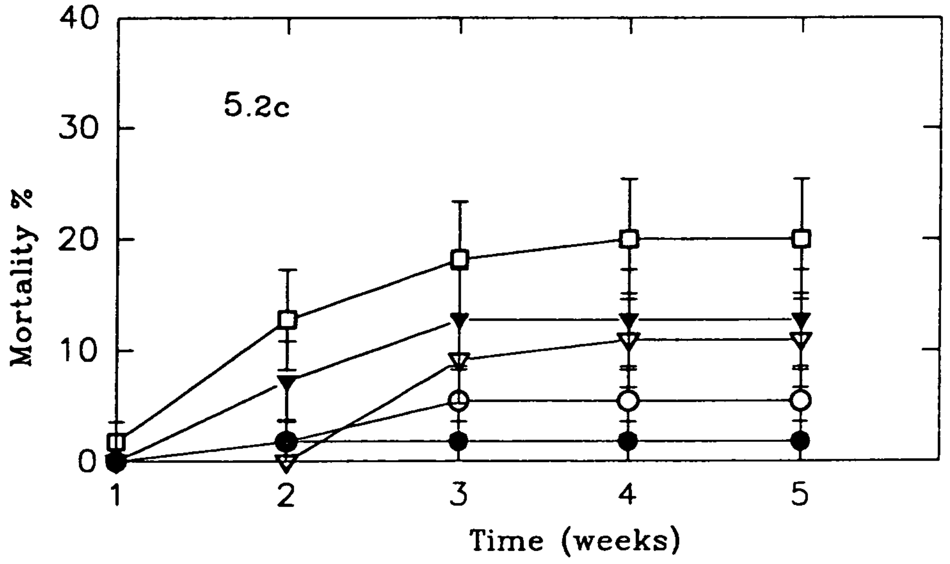
By the second week after insertion, a few cuttings produced shoots (2% in treatment 8 μg). The proportion of cuttings with shoots increased rapidly between weeks two and five (Figure 5.2e). In week three, the proportion of cuttings with shoots was significantly lower in the 40 μg treatment than in the others. In weeks four and five, there were no significant differences between the five treatments ($P > 0.05$; t-test), although the proportion of cuttings with shoots in the control treatment was relatively high.

Number of roots per rooted cutting

The mean number of roots per rooted cutting was significantly affected by variation in IBA concentrations by the fifth week. There was a general trend of increasing mean root number per rooted cutting with increasing quantities of auxin (Figure 5.2f). Treatment 250 μg was significantly higher ($P < 0.05$; t-test) than treatments 0 μg, 8 μg and 40 μg, but did not differ significantly ($P > 0.05$; t-test) from treatment 200 μg in terms of mean root number per rooted cutting. Similarly, treatment 200 μg was significantly higher ($P < 0.05$; t-test) than treatments 0 μg and 8 μg, but did not differ significantly ($P < 0.05$; t-test) from treatment 40 μg in this variable. There were no significant differences between treatments 0 μg, 8 μg and 40 μg. At the end of the five weeks of this experiment, 6.8, 7.5, 8.8, 11.3 and 13.8 mean root numbers per rooted cutting were recorded in treatments 0 μg, 8 μg, 40 μg, 200 μg and 250 μg of IBA respectively (Figure 5.2f).


5.2a
Rooting %
100
75
50
25
0
1
2
3
4
5


52.b
Callus formation %
100
75
50
25
0
1
2
3
4
5


5.2c
Mortality %
40
30
20
10
0
1
2
3
4
5
Time (weeks)

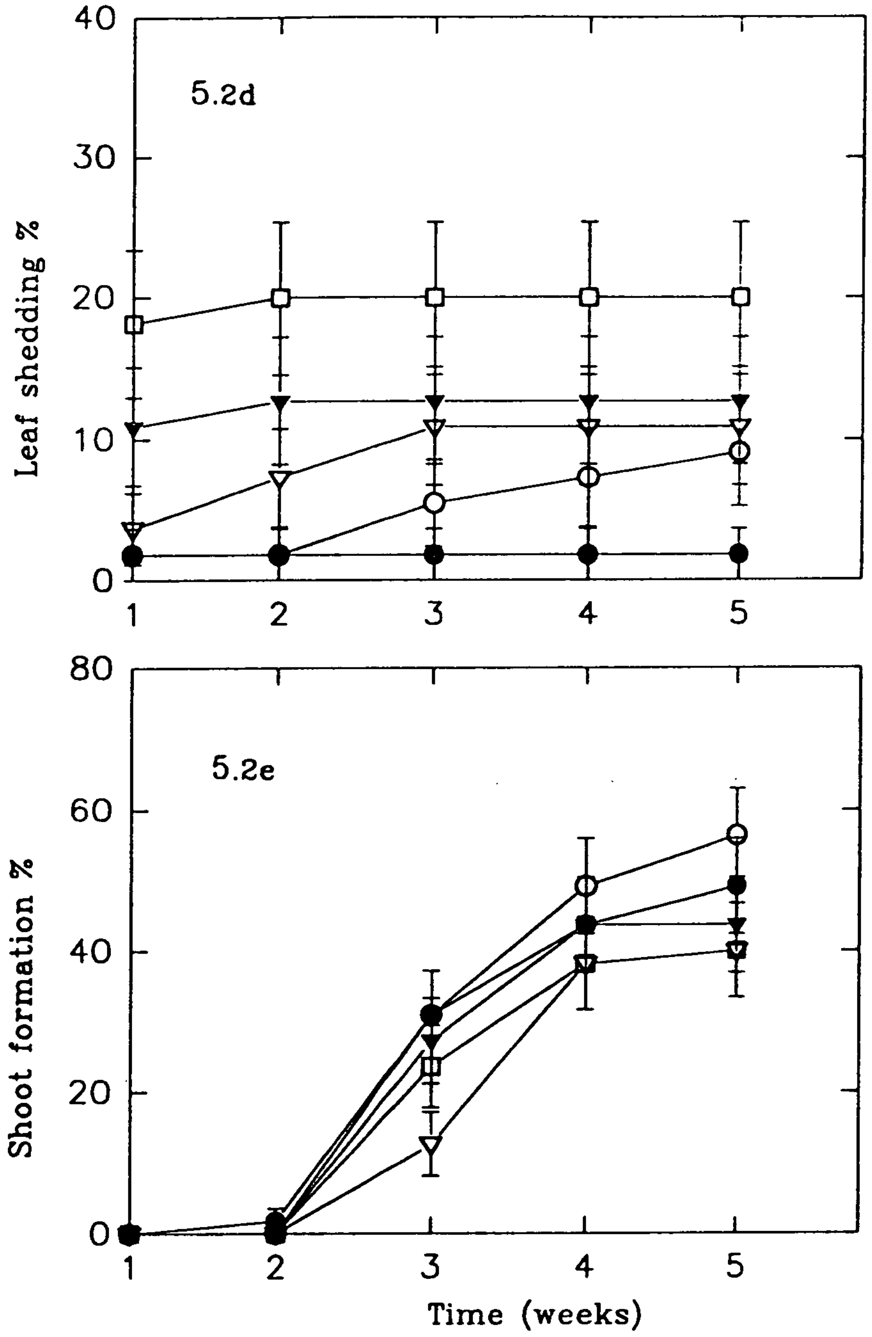


Figure 5.2: Effect of auxin (IBA) concentration on rooting of leafy stem cuttings of *G. africanum*, (○ = 0 µg, ● = 8 µg, ▽ = 40 µg, ▼ = 200 µg and □ = 250 µg). Bar = ± SE. (n = 55).

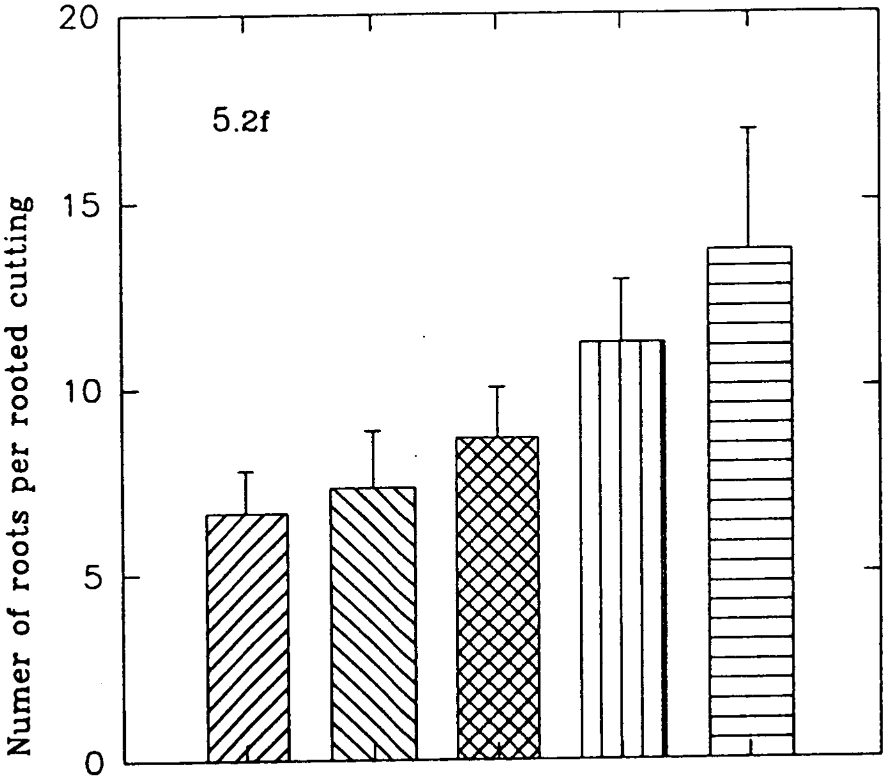


Figure 5.2f: Effect of auxin (IBA) concentration on root number per rooted cutting of *Gnetum africanum*. (▨ = 0 µg, ▧ = 8 µg, ▩ = 40 µg, ▥ = 200 µg and ▤ = 250 µg) (after 5 weeks). Bar = + SE. (n = 55).

5.4.0 Experiment 3: Effect of leaf area on rooting of leafy stem cuttings of *Gnetum africanum*

5.4.1 Introduction

Leaf retention has been found to have a stimulatory effect on the rooting of cuttings in many species (Leakey *et al.*, 1982b; 1990; Tchoundjeu, 1989; Reuveni and Raviv, 1981; Newton *et al.*, 1992b) (for details, see chapter one). Few such studies have been carried out on tropical plant species in general, and no previous investigations have been undertaken with *Gnetum africanum.* The present experiment was designed to investigate the effects of five leaf area treatments on rooting, leaf area being one of the major factors that influences rooting of leafy cuttings (Leakey, 1982b; Geary and Harding, 1984).

5.4.2 Materials and methods

Fifty vines of *Gnetum africanum* were selected from collection areas in the Southern Bakundu Forest Reserve on the 4th March 1993. Since the same number of vines were not collected from each area (in some areas only one vine qualified for selection), vines were not identified with particular collection areas in this experiment. Five cuttings from each vine were randomly assigned to each of five treatments. The five treatments were 0 cm^2, 12.5 cm^2, 25 cm^2, 50 cm^2 and 80 cm^2 leaf area obtained using graph paper. Each cutting was then treated with 250 μg of IBA and inserted randomly, but recording node position, into one of five lines in a block in the same propagator. There were five lines corresponding to the five vines and five cuttings per line corresponding to the five treatments, resulting in twenty-five cuttings (A, B, C, D, E) in one block. The rooting medium used in this experiment was sawdust. In total there were ten randomized blocks in the same propagator. The cuttings were assessed on a weekly basis as described earlier.

Analysis

Analysis of variance using SAS (1980) and a stepwise regression procedure 5 (Payne *et al.*, 1987) were applied to determine the influence of different leaf areas, and block, node position, stem length and leaf area on the rooting ability of the cuttings of *G. africanum* as described in Chapter 3

5.4.3 Results

Rooting percentage

The leafless cuttings in the control treatment did not root. However, rooting commenced in the leafed cuttings in week four, when the percentage of rooted cuttings was significantly lower ($P < 0.05$; t-test) in the 12.5 cm^2 leaf area treatment than it was in the 25 cm^2, 50 cm^2 and 80 cm^2 treatments, which were, in turn, not significantly different from each other ($P > 0.05$; t-test). This was maintained to the end f the experiment in week six, when the lowest (40%) and highest rooting percentage (86%) were recorded in the 12.5 cm^2 and the 80 cm^2 treatments respectively (Figure 5.3a). Analysis of variance of the last assessment at week six showed that there were significant differences between the five treatments, including the control ($P < 0.01$) (Table A17).

By the stepwise regression analysis, it was found that leaf area significantly affected the rooting ability of the cuttings of *G. africanum*. But the rooting ability of the cuttings of this species was not significantly affected by stem length, stem diameter at the base, block and node position at the end of the experiment in six weeks (Table A18).

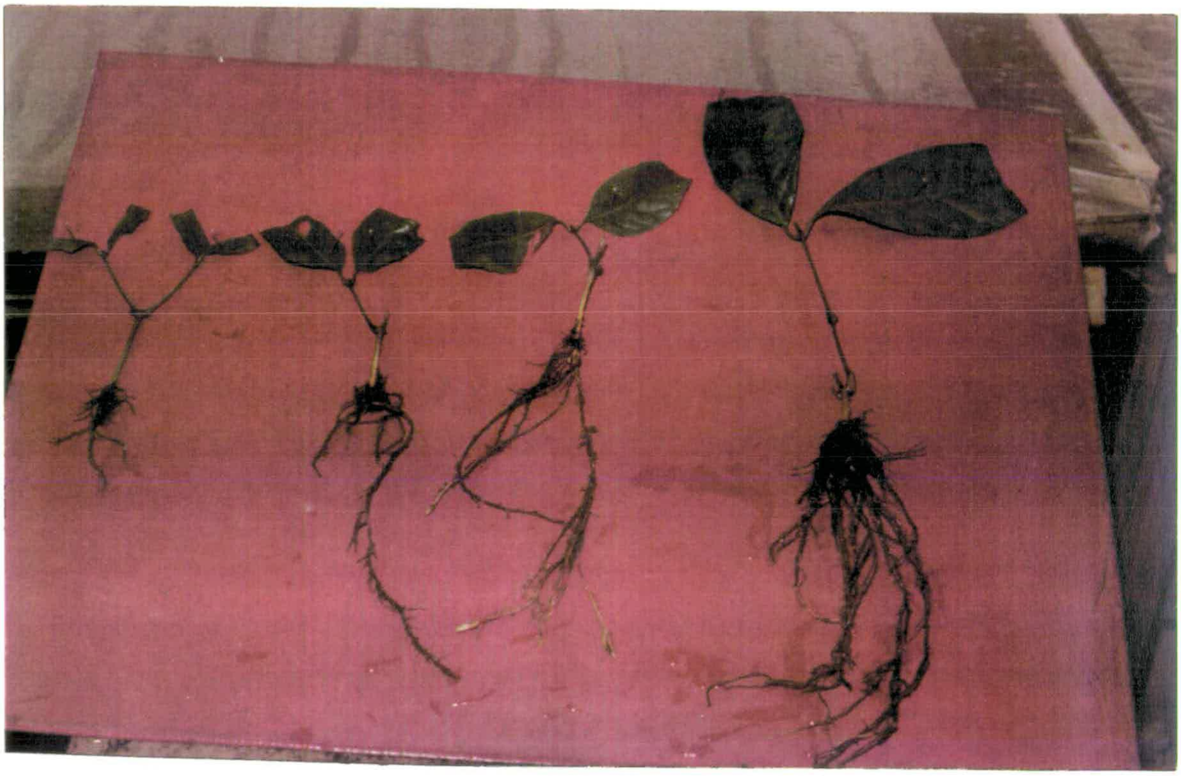


Plate 5.2: Rooted cuttings of *Gnetum africanum* exhibiting an increasing trend in root number per rooted cutting with increasing leaf area.

Callus formation

Cuttings started callusing after week three in treatments 25, 50 and 80 cm^2, which were not significantly different ($P > 0.05$; t-test) from each other, but significantly higher ($P < 0.05$; t-test) than the 12.5 and 0 cm^2 leaf areas. Percentage callus formation was significantly higher in cuttings with the 12.5 cm^2 than in those with the 0 cm^2 leaf area treatment ($P < 0.05$; t-test). Leafless cuttings were found to callus from week five, when 2% of the cuttings callused. Percentage callus formation in this treatment increased to 6% at the end of the experiment in week six.

Cutting mortality

By week two, no cutting had died in the leafless and the leafed area treatments. Percentage mortality increased between weeks two and three, when the proportion (38%) of dead cuttings was significantly higher ($P < 0.05$; t-test) in the leafless treatment than in leafed treatments. However, treatment 12.5 cm^2 had lost the highest proportion of 10% cuttings than the other three leafed treatments. By week four, percentage cutting mortality (2%) was significantly lower ($P < 0.05$; t-test) in treatment 80 cm^2 than in the other three leafed treatments, which did not differ significantly ($P > 0.05$; t-test) from each other, while 62% of the leafless cuttings had died. Percentage mortality then increased gradually to the final week, when the 12.5 cm^2 treatment, although lower than the leafless control, was higher than the other leafed treatments, which were not significantly different from each other ($P > 0.05$; t-test; Figure 5.3c).

Leaf shedding

The proportion of cuttings which lost their leaves tended to increase with increasing leaf area, although the rate of leaf shedding varied between the treatments. At the end of week two, 6% of the cuttings in the 12.5 cm^2 treatment had shed their leaves, whilst all the cuttings in the 80 cm^2 treatment retained their leaves until week four when leaves of 10% of the cuttings were abscessed. From weeks four to six, the percentage of cuttings which had shed their leaves was significantly higher in the 12.5 cm^2 leaf area treatment than in the other treatments ($P < 0.05$; t-test). The lowest percentage of leaf shedding was associated with the highest leaf area of 80 cm^2, although percentage leaf loss in this treatment was not significantly different ($P > 0.05$; t-test) from treatments 25 and 50 cm^2 leaf areas in weeks five and six (Figure 5.3d).

Shoot formation

In this experiment, shoots did not develop on the cuttings in any treatment in the first four weeks after insertion. In week five, 2% and 6% of the cuttings in treatments 12.5 and 50 cm^2 leaf areas respectively had produced new shoots, although there was no significant difference ($P > 0.05$; t-test) between these two treatments at this time. However, by week six, cuttings in all treatments had produced shoots, with a maximum of 12% released in the 0 cm^2 treatment (Figure 5.3e). Shoot formation in the 12.5 and 50 cm^2 treatments was significantly higher ($P < 0.05$; t-test) than in the 25 and 80 cm^2 treatments, but did not differ significantly from each other.

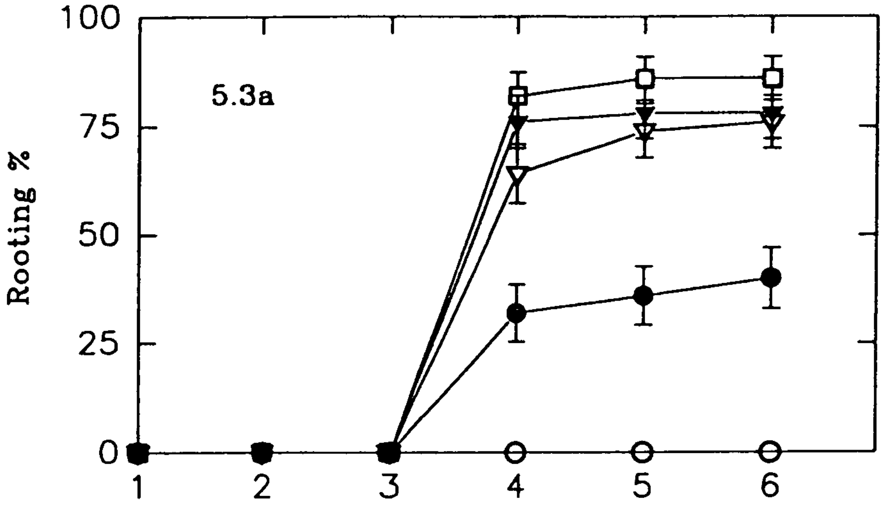
Number of roots per rooted cutting

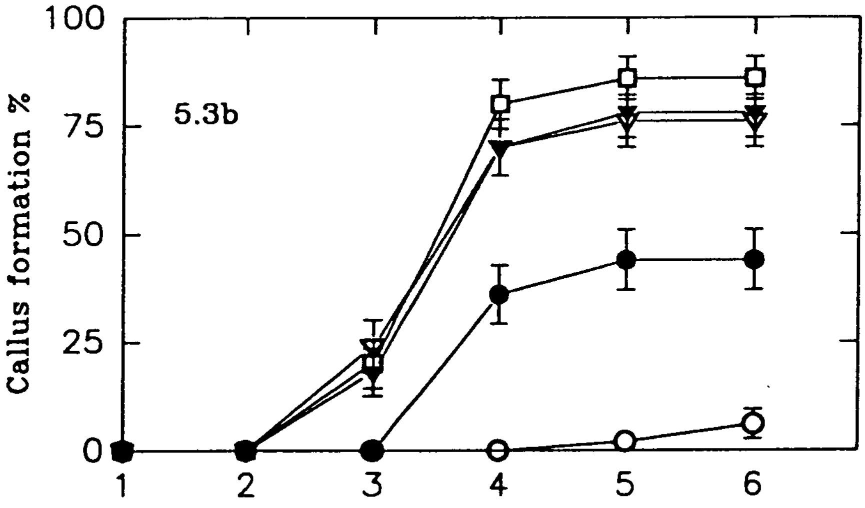
Leafless cuttings of this species did not root in this experiment. In the leafed cuttings, root number per rooted cutting tended to increase with increasing leaf area at week six after insertion (Figure 5.3f). The highest number of roots per rooted cutting was associated with treatment 80 cm^2, which was significantly higher ($P < 0.05$; t-test) than the other three leafed treatments. The number of roots was significantly higher in the 50 cm^2 treatment than in the 12.5 and 25 cm^2

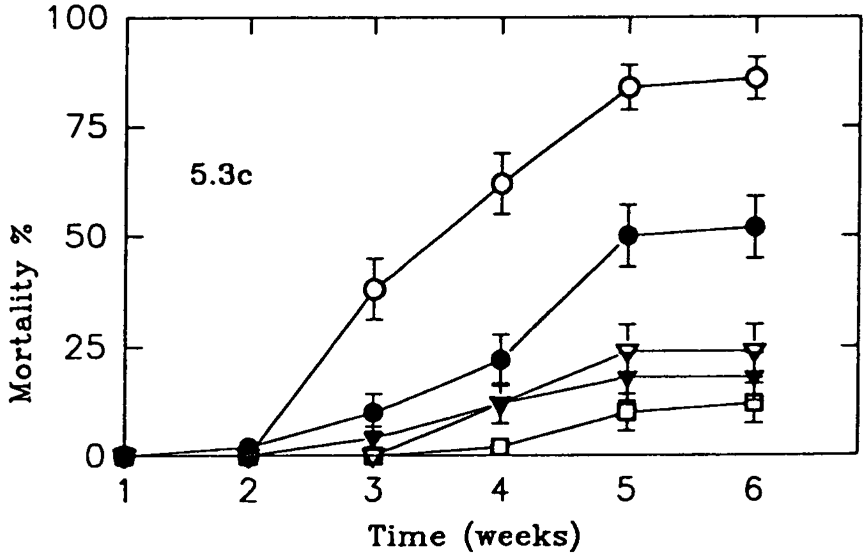
treatments, which did not in turn differ significantly from each other (*P* >0.05; t-test). There were many more roots per rooted cutting in *G. africanum* than in the other two species.

5.5 Discussion

Experimental evidence has shown that rooting media, auxin and the presence of leaves on cuttings play a central role in the initiation and development of adventitious roots in the leafy stem cuttings of tropical and temperate plants (Park and Andersen, 1989; Hartmann and Kester, 1983; Andersen, 1986; Jarvis, 1986; Haissig, 1986; Uniyal *et al.*, 1993; Leakey *et al.*, 1993; Newton *et al.*, 1992b). In the present study, it was found that in terms of the percentage rooting, cuttings treated with auxin were higher than the controls (though not significantly), those with higher leaf areas were higher than the leafless cuttings and in the media experiment the percentage of rooted cuttings ranged from 43% to 82% between the lowest and the highest media (a mixture of medium sand and sawdust and sawdust alone). This demonstrates that these factors have considerably enhanced the rooting ability of the cuttings of *G., africanum*, as has been found in many other tropical forest species (Leakey *et al.*, 1982b; Leakey *et al.*, 1990; Leakey *et al.*, 1994). However, the rate of rooting varied between the three experiments, with the cuttings in the media experiment starting to root in the third week, those in auxin experiment in second and those in the leaf area experiment in the fourth week after insertion (Figure 5.1a, 5.1b and 5.1c). At the end of each experiment, the highest percentage of rooted cuttings was 82% 70.9% and 86% in experiments 1, 2 and 3 respectively. These rooting results indicate that a combination of the optimum treatments could result in consistently successful rooting of this species in a cloning programme using vegetative propagation techniques.


5.3a
Rooting %
100
75
50
25
0
1
2
3
4
5
6


5.3b
Callus formation %
100
75
50
25
0
1
2
3
4
5
6


5.3c
Mortality %
100
75
50
25
0
1
2
3
4
5
6
Time (weeks)

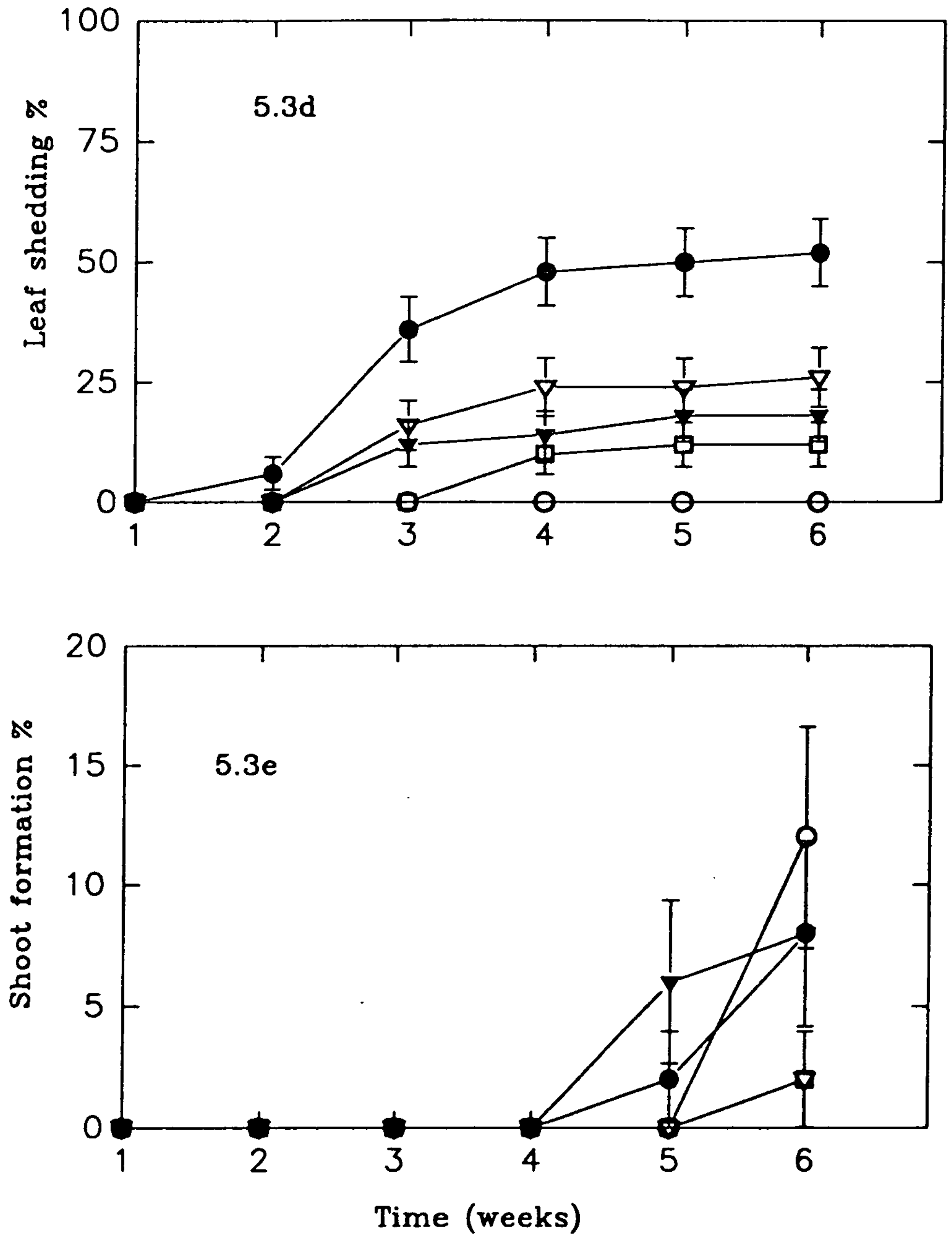


Figure 5.3: Effect of leaf area on rooting of leafy stem cuttings of *Gnetum africanum*, (○ = 0 cm^2, ● = 12.5 cm^2, ▽ = 25 cm^2, ▼ = 50 cm^2 and □ = 80 cm^2). Bar = ± SE. (n = 50).

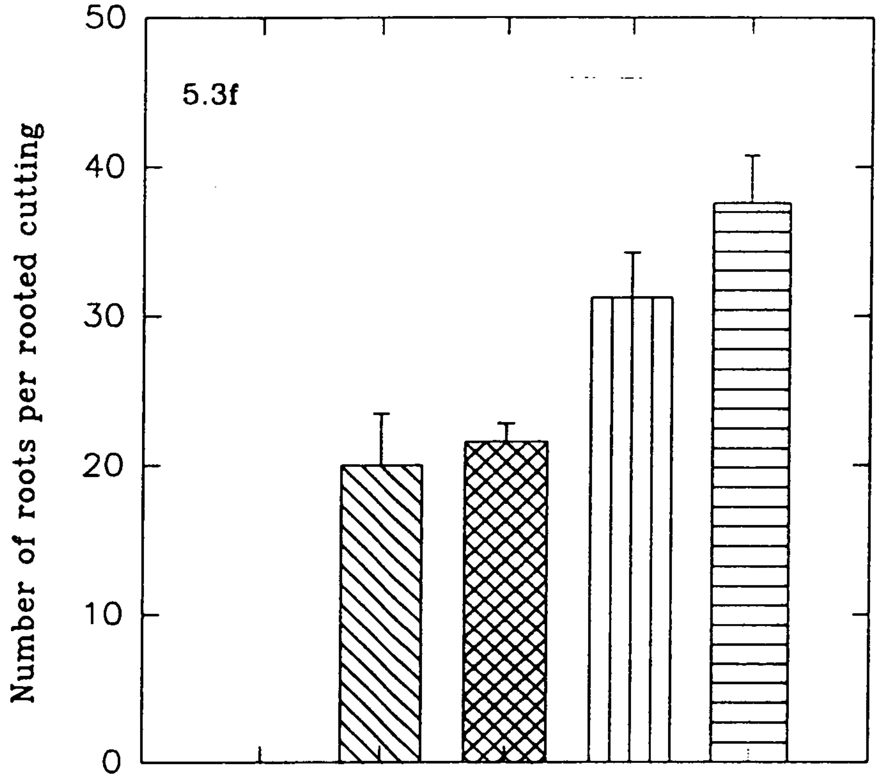


Figure 5.3f: Effect of leaf area on number of roots per rooted cutting of *Gnetum africanum*. (▧ = 12.5 cm^2, ▩ = 25 cm^2, ▥ = 50 cm^2 and ▤ = 80 cm^2) (after 6 weeks). Bar = + SE. (n = 50).

Percentage rooting

In the media experiment, the percentage rooting response of the cuttings of *G. africanum* to the six different media tested was found to be significant (*P* <0.05; Table A13) at the end of the experiment at week eight. This is consistent with other tropical species (Leakey *et al.*, 1994), most of which have been found to root differently in different rooting media. This also agrees with the observation of Hartmann and Kester (1983) that cuttings of some plant species may be greatly influenced by the kind of rooting medium used, both in the percentage and in the quality of roots produced. Some of these species differences were discussed in Chapter 3.

The highest percentage of rooted cuttings in this experiment was recorded in sawdust (82%) followed by fine sand 71%, although these two treatments were not significantly different (*P* >0.05) from each other (Table A13). This is in accordance with results obtained from *R. heudelotii* and *I. gabonensis* where the highest percentages of rooted cuttings were registered in sawdust and the second highest values were recorded in fine sand in each case. These results demonstrate that these three species, in terms of the percentage of rooted cuttings in sawdust, are similar to *Milicia excelsa* (Ofori, 1994) and *Gmelina arborea* (Leakey *et al.*, 1990), but contradict Mesén (1994), who recorded the lowest percentage of the rooted cuttings of *Cordia alliodora* in sawdust (see discussion in Chapter 4). In addition these results do not conform with those of O'Dell and Stoltz (1978) who registered a percentage mean value of (82%) of the rooted cuttings of three species of *Juniperus horizontalis* (Plumosa), *Pyracantha* (Shawnee) and *Hedera helix* in medium sand. The variation displayed by these species among different rooting media is in agreement with the observation made by Loach (1985) that the optimum rooting medium differs between species, and that even with related cultivars in the same genus, divergent results can be obtained in different media.

Rooting media have been shown to influence the rooting ability of leafy stem cuttings in various ways (O'Dell and Stoltz, 1978; Loach, 1985; Andersen, 1986; Hartmann and Kester, 1983). The failure or success of cuttings to rooting in certain media has been attributed to the physical properties of such media (Park and Andersen, 1986; Loach, 1985; O'Dell and Stoltz, 1978). Medium porosity, oxygen diffusion rate, water retention capacity and pH have all been implicated in the rooting process of leafy stem cuttings (Andersen, 1986; Hartmann and Kester, 1983). Optimum pore-space in a rooting medium allows easy adequate oxygen diffusion to the cutting base to facilitate tissue respiration, which creates a basipetal sugar concentration gradient at the rooting zone (Dick *et al.*, 1994). An inappropriate air:water ratio in a rooting medium may have an adverse effect on rooting. Waterlogging may hinder oxygen diffusion to the cutting base since air it has been shown to be 10,000 times slower in water than aerated rooting medium (Loach, 1985). There has been experimental evidence that the pH of a rooting medium may inhibit rooting and promote callusing at low values, moderate callusing and enhance rooting at neutrality and inhibit both callusing and rooting at values higher than 7.5 (Hartmann and Kester, 1983). The particle size of the medium in use, pathogen infection and microclimate fluctuation may cause rooting variation in cuttings (O'Dell and Stoltz, 1978; Hartmann and Kester, 1983; Howard, 1965). In the present study, the high percentage of rooted cuttings in sawdust and fine sand might have been due to their relatively high air:water ratios.

There were no significant differences between the five auxin (IBA) treatments in experiment 2, where the percentage of rooted cuttings ranged from 51% to 71% between treatments 0 μg (control) and 250 μg IBA. The fact that the percentage of rooted cuttings was above 50% and did not differ significantly from the treated cuttings demonstrates that *Gnetum africanum* is similar to *R. heudelotii*, and to other tropical species such as *Nauclea diderrichii* (Leakey, 1994) and *Prosopis juliflora* (Dick *et al.*, 1991), which were all successfully rooted by leafy stem cuttings without auxin application. Successful rooting of some plant species without auxin application was discussed in Chapter 3. In addition, the fact that the percentage of rooted cuttings increased with increasing IBA concentrations indicates that the optimum dose above which this auxin can be toxic to ***Gnetum africanum*** was not attained in this experiment. Similar observations were made on the cuttings of ***Nauclea diderrichii*** (Leakey, 1991) and ***Cordia alliodora*** (Mesén, 1994), where the percentage of rooted cuttings attained 100% in the former species irrespective of auxin concentration and 70% in the latter, although the highest IBA dose for the two species was 160 μg in *N. diderrichii* and 40 μg in *C. alliodora* as against 250 μg applied to the cuttings of *G. africanum* in this experiment.

Varying rooting responses of the leafy stem cuttings of many plant species to varying auxin doses may be ascribed the endogenous auxin status of such cutting at the time of severance (Torrey, 1986; Weigel *et al.*, 1984). Applied auxin may promote rooting by mobilizing carbohydrates to the rooting zone, enhancing the activity of hydrolytic enzymes and facilitating the synthesis of endogenous auxin and auxin co-factors (Nanda *et al.*, 1968a; Rana *et al.*, 1987; Middleton Jarvis, and Booth, 1980) (see Chapter 4).

The leafless cuttings of *G. africanum* in experiment 3 failed to produce roots, as recorded previously with *R. heudelotii* and *I. gabonensis*. Similarly, leafless cuttings of *Terminalia spinosa* failed to root (Newton *et al.*, 1992b), although the leafless cuttings of some other species have been found to root (Hamzah, 1992; Leakey 1982b) (see Chapter 4). The failure to root in leafless cuttings may be attributed to their inability of photosynthesize during propagation (Newton *et al.*, 1992b), lack of leaf surface for transpiration and carbon dioxide uptake, and perhaps reduced auxin production (Middleton, Jarvis and Booth, 1980) (see Chapter 4) in the leafless cuttings.

There were significant differences (P <0.001) between the four leaved treatments in this trait at the end of the experiment in week six, with a percentage range of 40% to 86% recorded in the 12.5 cm^2 and 80 cm^2 treatments respectively (Table A17). This contrasts with *Nauclea diderrichii* (Leakey, 1991), where, except for the 5 cm^2, the percentage of rooted cuttings did not differ significantly between the four leaved treatments of 5 cm^2, 10 cm^2, 50 cm^2 and 100 cm^2. The increasing trend in the percentage of rooted cuttings with increasing leaf area (Figure 5.3a) conforms with the results of *R. heudelotii* and *I. gabonensis* in this study, and those of *E. camaldulensis* (Geary and Harding, 1984). The percentage increase of the rooted cuttings of *G. africanum* in response to increasing leaf area suggests that the optimum leaf area for the rooting of the cuttings of this species may be higher than those tested in this experiment (Plate 5.2), in accordance with the observations made on *R. heudelotii* and *I. gabonensis*. Some tropical plant species have been found to exhibit optimum leaf area for maximum root production (Leakey *et al.*, 1982b; Asanga, 1989; Tchoundjeu, 1989), while others have displayed no tendency towards an optimum leaf area for maximum rooting (Reuveni and Raviv, 1981; Newton *et al.*, 1992b; Dick *et al.*, 1994) (see Chapter 4).

There is experimental evidence to the effect that leaf retention and optimum leaf area on a cutting may reflect a balance between the processes of photosynthesis and transpiration, thereby enhancing rooting in leafy stem cuttings (Leakey and Coutts, 1989). Production of current assimilates in the process of photosynthesis and transportation of carbohydrates basipetally to the rooting zone, has been shown to be crucial for adventitious root formation in a range of species (Reuveni Raviv, 1981; Newton *et al.*, 1992b; Dick *et al*, 1994) (see discussion Chapter 4).

Callusing

While the rate of callus formation was similar in experiments 1 and 3, all starting in the third week after cutting insertion, that of experiment 2 was different, commencing in the second week of the experiment (figures 5.1b, 5.2b and 5.3b). In addition, the proportion of callused cuttings in experiment 2 in the third week was much higher (87%) than it was in experiments 1 and 3, where the highest values of 5% and 24% were recorded respectively. By the end of each experiment, the highest percentage of callused cuttings was 86%, 98% and 86% associated with experiments 1, 2 and 3 respectively. The percentage of rooted cuttings was similar to that of callused cuttings in the media, auxin and leaf area experiments of this chapter (82.1%. 71% and 86.%, respectively). These results show that 82% of 86% callused cuttings rooted in experiment 1, 71% of 98% callused cuttings rooted in experiment 2 and all the 86% callused cuttings in experiment 3 rooted. It would appear that the pH of the medium in experiment 3 was optimum (6.5 to 7.5), thereby moderating the mass of callus production and enhancing root formation (Hartmann and Kester, 1983; Komissarov, 1969).

In experiment 1, the highest percentage of callused cuttings was recorded in sawdust, followed by fine sand, while the lowest was registered in gravel. This pattern of callus formation is similar to that of root formation, where the highest percentage of rooted cuttings was associated with sawdust, followed by fine sand and the lowest percentage of rooted cuttings was recorded in gravel. This demonstrates that the percentage of callused cuttings is positively related to that of rooted cuttings in *G. africanum*. This is in contrast with *I. gabonensis*, where callus formation was not related to root production, neither of which was affected by any of the six media, when the rooting data was analyzed statistically using ANOVA (SAS, 1980).

In the auxin experiment, the highest percentage of callused cuttings was recorded in the 8 μg IBA treatment, while the lowest was recorded in the highest auxin concentration of 250 μg at the end of the experiment. This is in contrast with the percentage of rooted cuttings, where the highest percentage was recorded in the highest auxin concentration. This indicates that in this experiment, high auxin concentrations enhanced callus production, while low doses promoted rooting. This is in accordance with the hypothesis that callus formation is independent of root production (Hartmann and Kester, 1983; Van der Lek, cited by Komissarov, 1969). However, these results contradict those of *R. heudelotii* where the highest percentage of callused and rooted cuttings were recorded in the 8 μg treatment.

The percentage of callused cuttings in all the treatments including the control ranged from 80% to 98%, suggesting that *G. africanum* is similar to *Dalbergia sericea* (Uniyal *et al.*, 1992), who reported that callus formation was seen on cuttings of all the auxin treatments. However, while a high proportion of the untreated cuttings of this species callused and rooted, the control cuttings of *Dalbergia sericea* callused but failed to root.

Although the physiology of callus has not been sufficiently studied (Komissarov, 1969), it is believed that callus enhances the supply of water to the cuttings during propagation (Flerov and Kovalenko, 1952, cited by Komissarov, 1969). Massive callus formation in some species may tend to inhibit root formation (Park and Andersen, 1989).

Mortality

The time when cutting mortality started between the three experiments varied considerably, such that by week one 2% of the cuttings in experiment 2 had died. However, cutting mortality did not start in experiments 1 and 3 until week three when 7% and 38% of the cuttings in these experiments died respectively. By the end of each experiment, the highest percentages of dead cuttings in the three experiments were 48%, 20% and 52% respectively. The high mortality values recorded in experiments 1 and 3 demonstrates how sensitively the cuttings of this species respond to treatment changes in media and leaf area during propagation. Other factors such as seasonal variation in rooting may also account for variation between experiments. This indicates that these two factors are more limiting than auxin in the rooting of leafy stem cuttings of *G. africanum*, in agreement with other tropical species (Leakey *et al.*, 1994). This is confirmed by the fact that most tropical species have necessarily had to be rooted in a medium in the presence of a leaf for rooting to be achieved, whereas some have been successfully rooted without auxin application (Leakey *et al.*, 1990; Newton *et al.*, 1992b; Reuveni and Raviv, 1981; Hamzah, 1992).

At the end of experiment 1 in week eight, the highest and lowest proportions (48% and 13%) of dead cuttings were recorded in the mixture of medium sand and sawdust and sawdust treatments respectively. In this respect, *Gnetum africanum* is similar to *R. heudelotii* and *I. gabonensis*, where the lowest percentage of dead cuttings was recorded in sawdust, although the highest proportion of dead cuttings was associated with different rooting media. Similarly, the highest survival of the cuttings of *Milicia excelsa* was registered in sawdust in a media experiment (Ofori, 1994). But this is contrary to *Cordia alliodora*, where the lowest survival was recorded in sawdust (Mesén, 1994). These results demonstrate that the survival of different plant species varies with different rooting media.

The cuttings of *G. africanum* were observed to die mostly by rotting after dropping the leaves. It was not easy to tell whether rotting started from the top or bottom since the whole stem length was affected, indicating that the rotting started from the pith within the cuttings and then spread to the outer parts. This might have been caused by waterlogging, bacterial or fungal infection, xylem blockage, carbohydrate and/or starch saturation at the cutting base, inadequate auxin activity or a lack of oxygen for tissue respiration at the cutting base (Hartmann and Kester, 1983; Mujib, 1993; Leakey and Coutts, 1989; Haissig, 1986; Dick *et al.*, 1994). (see discussion in Chapters 3 and 4).

In experiment 2, cutting mortality started in week one and increased rapidly to the second week when the rate of increase was much lower until the end of the experiment at week five (Figure 5.2c). Except for the control treatment (0 μg), the percentage of cutting mortality increased with increasing auxin concentration, such that by the end of the experiment, the highest proportion (20%) of dead cuttings was recorded in the 250 μg treatment, while the lowest (2%) was recorded in 8 μg treatment. In this experiment, the percentage of rooted cuttings was also positively related to auxin concentration, suggesting that most of the cuttings which survived in this treatment rooted, while most of the cuttings which survived in the other treatments did not root.

The relatively high and low mortality values and the relatively high and low percentage rooting of the cuttings of *G. africanum* at the auxin concentrations of 250 μg and 200 μg (Figures 5.2a and 5.2c) demonstrate that the optimum IBA concentration for this species may lie between 150 μg and 250 μg. It would appear that at the highest concentration of 250 μg, IBA had become slightly toxic to the cuttings, although it enhanced the rooting of those that survived, in accordance with Middleton, Jarvis and Booth (1978), who observed that high doses of auxin induced phytotoxicity in the cuttings of mung bean seedlings. The different ways in which auxin could have caused mortality were discussed in Chapters 3 and 4.

In the leaf area experiment, cutting mortality commenced in week two, when 2% of the cuttings in 12.5 cm^2 had died. Contrary to expectation, none of the leafless cuttings had died by this time. However, by the end of the experiment in week six, all the leafless cuttings had died, while percentage mortality among the leafed treatments ranged from 12% to 52% in the 80 cm^2 and 12.5 cm^2 leaf area treatments respectively. The percentage of cutting mortality was inversely related to leaf area, which was in turn positively related to the percentage of rooted cuttings (Figures 5.3a and 5.3c). In this regard, *G. africanum* is similar to *R. heudelotii*, where there was an inverse relationship between cutting mortality and leaf area.

The inverse relationship between cutting mortality and increasing leaf area observed in this experiment may be ascribed to the fact that current photosynthate production was higher in the larger leaf areas than in the smaller ones. This might have provided more current assimilates for respiration, and perhaps reduced the incidence of cutting mortality.

Leaf abscission

The rate of leaf abscission in the three experiments varied considerably, such that 18% of the cuttings in experiment 2 lost their leaves in the first week, while those in experiments 1 and 3 did not start leaf abscission until weeks four and two respectively. By the end of each experiment, the highest proportion of 48%, 20% and 52% of the cuttings had shed their leaves in experiments 1, 2 and 3 respectively. There were more variations in the percentage of leaf abscission in experiments 1 and 3, where leaf loss ranged from 13% to 48% and 12% to 52% respectively than in experiment 2, where the range was between 2% and 20% (Figures 5.1d, 5.2d and 5.3d). While the percentage of leaf abscission was negatively related to the percentage of rooted cuttings in experiments 1 and 3, there was an inverse relationship between these two variables in experiment 2, where the highest percentage rooting and that of leaf abscission were recorded in the 250 μg IBA concentration.

At the end of the media experiment in week eight, the highest proportion of cuttings that had shed their leaves was recorded in the mixture of medium sand and sawdust medium, while the lowest was associated with the sawdust medium, in which the highest percentage of rooted cuttings of this species was also recorded. This is in contrast with *R. heudelotii* and *I. gabonensis* where the highest percentage of leaf abscission was recorded in the mixture of gravel and sawdust and in the gravel media respectively.

Leaf abscission in cuttings may be attributed to water stress which could result from the low water content of the medium (Gislerod, 1983), or from waterlogging, which could cause anoxia leading to leaf shedding (Loach, 1985) (see discussion in Chapter 4).

At the end of experiment 2 in week five, the percentage of leaf abscission ranged from 2% to 20.0% in the 8 μg and 250 μg treatments respectively, while 9%, 4% and 13% leaf losses were associated with treatments 0 μg (the control), 40 μg and 200 μg respectively. The lowest percentage of cutting mortality was also recorded in this treatment (8 μg). In this connection, *G. africanum* is similar to *I. gabonensis* where the lowest cutting mortality and leaf abscission were both recorded in the 8 μg auxin treatment. These results are not consistent with those of *R. heudelotii* where the highest leaf abscission, percentage of rooted and callused cuttings were all registered in the 40 μg treatment. The fact that the highest percentage of leaf abscission and that of rooted cuttings were both recorded in the highest auxin concentration (250 μg) suggests that the optimum auxin concentration for this species lies between 150 μg and 250 μg (see earlier discussion). Consequently, the 250 μg dose might have been toxic to the cuttings resulting in leaf shedding. Higher auxin doses than this may lead to more leaf abscission.

In experiment 3, leaf abscission started in week two and increased rapidly to week four. From here onwards, the increase was more gradual until the last assessment, when the values ranged from 12% to 52% in treatments 80 cm^2 and 12.5 cm^2 respectively. Leaf abscission tended to increase with decreasing leaf area. These results are in accordance with those of *R. heudelotii* and *I. gabonensis*, where percentage leaf abscission was inversely related to leaf area, but contradict those of *Terminalia spinosa* (Newton et al., 1992b) and those of *Khaya ivorensis* (Asanga, 1989), where larger leaf areas tended to abscise more than smaller leaf areas (see discussion in Chapters 3 and 4). Higher percentage leaf shedding in the smaller leaf areas could be attributed to the inability of the smaller leaf areas to attain an optimum balance between transpiration losses and photosynthesis (Leakey and Coutts, 1989). The highest leaf shedding was found to be associated with the 12.5 cm^2 leaf area treatment in which the highest cutting mortality was recorded. In view of this, *G. africanum* is similar to *I. gabonensis* where both the highest cutting mortality and leaf abscission were recorded in the 12.5 cm^2 leaf area treatment.

Shoot formation

The time at which cuttings formed shoots in the three experiments differed considerably, such that by week two 1% of the cuttings in the auxin (IBA) experiment had formed shoots, while shoot formation did not occur in the leaf area experiment until week five after cutting insertion. It was observed that no cutting in any of the six media in the media experiment formed shoots. The proportion of cuttings which formed shoots in experiments 2 and 3 increased rapidly to the last assessment in weeks five and six respectively. The percentage of cuttings that formed shoots ranged from 40% to 56% and from 2% to 12% in experiments 2 and 3 respectively. This indicates that the highest shoot percentages were formed in the auxin experiment.

In the media experiment, none of the cuttings in any of the six media produced shoots. The failure to produce shoots by the cuttings in any of these media, particularly in sawdust and fine sand where percentage rooting results were high, could be attributed to the carbohydrate reserves of the cuttings at the time of severance (Haissig, 1986). The cuttings were obtained from locations where harvesting was intensively carried out. It is possible that the carbohydrate contents of cuttings pre-severance might have been so low that the current assimilate was just enough for rooting. Insufficient may have been left for budbreak until rooting was complete (Okoro and Grace, 1976).

In experiment 2, shoot formation commenced in all the treatments at week three after cutting insertion and increased rapidly to the end of the experiment in week five, when the highest and lowest proportions of 56% and 40% of the cuttings produced shoots in treatments 0 μg (control) and 250 μg of IBA concentrations respectively. This demonstrates that the proportion of cuttings that produced shoots was higher in the control treatment than it was in the IBA treated cuttings. In this connection, *G. africanum* is similar to *I. gabonensis* where the highest proportion of cuttings that formed shoots was associated with the control (0 μg) treatment. The fact that the highest percentage of cuttings to form new shoots was recorded in the control treatment in which the lowest percentage of rooted cuttings was registered indicates that the carbohydrate reserves and the current assimilates of these cuttings might have been used in budbreak and shoot development to the detriment of root formation.

Factors such as carbohydrate reserves (Wright, 1975), current assimilate production (Newton *et al.*, 1992b) and leaf trimming (Geary and Harding, 1984) have been implicated in new shoot formation in cuttings during propagation (see discussion Chapters 3 and 4).

In experiment 3, new shoot formation did not start until the fifth week after cutting insertion. From week five, the percentage of shoots formed increased sharply such that at the end of the experiment in week six, the percentages of shoots formed in treatments 0 cm^2, 12.5 cm^2 and 50 cm^2 were significantly higher than those in treatments 25 cm^2 and 80 cm^2. The leafless cuttings formed the highest percentage of new shoots, although they failed to root. This is in accordance with Newton *et al.* (1992b) who reported that leafless cuttings of *Terminalia spinosa* sprouted and produced new leaves but did not root. These results are different to those of *R. heudelotii* and *I. gabonensis* where leafless cuttings neither rooted nor sprouted.

Root number

The mean number of roots per rooted cutting in all the three experiments in this chapter ranged from 13.3 to 26.5, 6.6 to 13.7 and 0 to 37.6 in experiments 1, 2 and 3 respectively. The highest mean number (37.6) of roots per rooted cutting among the three experiments was recorded in the leaf area experiment, where it was associated with the 80 cm^2 which was the highest leaf area. The highest mean number of roots per rooted cutting in experiments 1 and 2 were associated with the fine sand treatment and the 250 μg treatment respectively. The zero roots per rooted cutting was recorded in the leafless cuttings in experiment 3. The highest mean number of roots per rooted cutting associated with the 80 cm^2 leaf area and with the 250 μg, which are the highest treatments in each of the two experiments, is consistent with the observations on rooting percentage of *G. africanum* and with those on *R. heudelotii* and *I. gabonensis* where the highest mean number of roots per rooted cutting were associated with the highest leaf area (80 cm^2; Figures 3.3f. 4.3f 5.3f).

In experiment 1, the highest mean number of roots was recorded in fine sand. These results agree with those of other tropical species such as *Cordia alliodora* where the highest mean number of roots were recorded in fine sand (Mesén, 1994), but contradicts those of *R. heudelotii* and *I. gabonensis* in this study and those of *Milicia excelsa* (Ofori, 1994), where the highest mean number of roots per rooted cutting was registered in sawdust. The lowest mean number of roots per rooted cutting of this species was recorded in a mixture of gravel and sawdust, contrary to the to *R. heudelotii* and *I. gabonensis* where the mean number of roots per rooted cuttings were both recorded in gravel.

The response of *G. africanum* as expressed in this trait may be ascribed to air:water ratio and such other physical properties as particle size, porosity, aeration and water retention capacity of the rooting media (O,Dell and Stoltz, 1978; Loach, 1985; Hartmann and Kester, 1983) (see discussion in Chapter 3 and 4).

In the auxin experiment, mean number of roots per rooted cutting tended to increase with increasing IBA concentration. This concurs with the results of Leakey *et al.* (1982b) who found that the mean number of roots per rooted cutting of *Triplochiton scleroxylon* increased with increasing auxin concentration, and Bhatt and Todaria (1993) who reported the same trend in the cuttings of *Debregeasia salicifolia* and *Bohemeria regulosa* collected from altitudes of 1760 m and treated with different IBA concentrations, except the control where no cutting rooted.

The fact that the mean number of roots per rooted cuttings increased with increasing auxin concentration may be attributed to the relative efficiency of the increasing dosage in facilitating the synthesis of endogenous auxin, enhancing the activities of hydrolytic enzymes and mobilizing and transporting carbohydrates to the cutting base (the rooting zone) (Middleton, Jarvis and Booth, 1980; Nanda *et al.*, 1968; Rana *et al.*, 1987; Haissig, 1986) (see earlier discussion).

However, the mean number of roots per rooted cutting in the leafed treatments increased with increasing leaf areas (Figure 5.3f). In this connection, *G. africanum* is similar to *R. heudelotii* and *I. gabonensis*, where the mean number of roots per rooted cutting was positively related to the leaf area. This is in accordance with the results of Breen and roots per rooted cutting of *Prunus munsoniana* and *Prunus cerasifera* and increasing leaf number.

The highest percentage of rooted and callused cuttings were also recorded in the 80 cm^2 leaf area treatment. These observations are consistent with those of *I. gabonensis* and *R. heudelotii*, where the mean number of roots per rooted cutting and the percentages of rooted and callused cuttings were associated with the 80 cm^2 leaf area treatment. Seemingly, the leaf area above which the number of roots per rooted cutting may start to decline is greater than 80 cm^2. This may account for the observed increase in the mean number of roots per rooted cutting with increasing leaf areas.

CHAPTER 6

Effects of different stockplant management treatments on rooting of leafy stem cuttings of *Ricinodendron heudelotii*

CHAPTER 6

6.0 Effects of different stockplant management treatments on rooting of leafy stem cuttings of *Ricinodendron heudelotii*

6.1.0 Introduction

Ricinodendron heudelotii is a West African multipurpose tree species valued for its seeds and timber (see Chapter One). This study was aimed at increasing the understanding of physiological requirements prior to propagating it vegetatively with a view to facilitating domestication of the species.

Factors affecting stockplants and the subsequent rooting of cuttings were presented in Chapter One. In this chapter, experiments were designed to study the effect of three of these factors on rooting leafy stem cuttings of *R. heudelotii.* The experimental treatments included: (i) different stump heights, (ii) stockplant shading and (iii) fertilizer application.

6.2 Experiment 1: Effect of different stump heights on rooting of leafy stem cuttings of *Ricinodendron heudelotii.*

6.2.1 Introduction

Rooting ability of cuttings is influenced by the position from which they are taken on the stockplant (Leakey, 1983; 1985; 1989b; Brix, 1973; Bonga, 1981; Wiltshire & Reid, 1992; Leakey *et al.*, 1993; Fortanier & Jonkers, 1976). This variation can be attributed, among other factors, to the condition and age of the stockplant (Leakey *et al.*, 1992). A distinction between the age of the stockplant and that of the shoot tissues growing from it has been made by Leakey *et al.*, (1992), Barchert (1976), and

Fortanier and Jonkers (1976) who identified the former (ontogenetic age) with irreversible characteristics related to reproductive maturity, and the latter (chronological age) with reversible changes related to the physiology and morphology of the shoot tissues. The chronological age of shoots can be manipulated by pruning, hedging and coppicing in order to reduce morphological barriers to rooting such as lignification, secondary thickening and leaf senescence. Pruning may also enhance the availability of promotory factors for rooting, such as soluble carbohydrates, nutrients and growth regulators (Leakey *et al.*, 1992; Leakey, 1989b). Experimental evidence from *Triplochiton scleroxylon* suggests that there are physiological differences in shoots with the same chronological age, as expressed by their rooting ability (Leakey *et al.*, 1992).

The objective of the present experiment was to investigate the possibility of rooting reproductively mature cuttings to reduce the height and time to fruiting of clonal plants. In this regard, the effect of three different stump heights on rooting leafy stem cuttings of *Ricinodendron heudelotii* was studied.

6.2.2 Materials and methods

A number of plants from eighteen clones derived from previous experiments were planted in 1991 near the propagation unit. The plants were established in lines along the slope at a spacing of 1.5 m x 1.5 m, and were maintained by weeding and mulching when necessary for eleven months (Plate 6.1). On the 20th of April 1993, three trees were randomly selected from each of four clones (2001, 2032, 2015 and 2034) chosen from the lines and cut to three different heights of 0.3 m, 1.2 m and 2.1 m. By the 16th May 1993, four to seven nodal sprout shoots had been produced per stockplant (Plate 6.2). A total of four shoots were obtained from each stump. Each clone provided sixty cuttings (5 cuttings per shoot x 4 shoots per stump x 3 stumps per clone). Four blocks were established according to the maximum number of shoots obtained from one stockplant. Each cutting, after trimming the leaf to about

$50\ cm^2$ and applying $40\ \mu g$ of IBA to the base of each with a microsyringe, was inserted in the propagator in node order. The sixty cuttings from the twelve shoots filled three lines in each of the four blocks, bringing the total to two hundred and forty cuttings, all in the same propagator. From the second week, cuttings were assessed as described in earlier experiments.

Analysis

Analysis of variance and that of deviance by stepwise regression were carried out at the end of the experiment in week four to study the influence of the three different stump heights and the other factors investigated on the rooting ability of the cuttings of *R. heudelotii* as described in earlier experiments.

6.2.3 Results

Rooting percentage

Ten percent of the cuttings in the 0.3 metre treatment had rooted in the first week of the experiment, while cuttings from the other two height treatments (1.2 and 2.1 m) commenced rooting in the second week. Percentage rooting was significantly higher in treatment 0.3 m than in the other two treatments in the second, third and fourth weeks ($P < 0.05$; t-test) (Figure 6.1a), when rooting was complete. At the end of the experiment in week four, analysis of variance test confirmed that height treatment 0.3 m was significantly ($P < 0.001$) higher than the other two height treatments (1.2 m and 2.1 m) in terms of the percentage of rooted cuttings. However, the proportion of rooted cuttings in the 1.2 m treatment was slightly higher than that in the 2.1 m treatment, although there were no significant differences ($P > 0.05$) between these two treatments (Table A19).

Plate 6.1: Cloned stockplants of *Ricinodendron heudelotii* from earlier experiments growing in near the propagation unit in Kumba, Cameroon.

Plate 6.2: Three stump heights of *Ricinodendron heudelotii* stockplants cut to 0.3 m, 1.2 m and 2.1 m of the same clone in a line.

By a stepwise regression analysis, it was found that the rooting ability of the cuttings of *R. heudelotii* was significantly influenced by the three different stump heights and by cutting diameter at the cutting base, but block, clone and cutting length did not have a significant influence on the rooting ability of the cuttings of this species (Table A20).

Callus formation

The proportion of callused cuttings was significantly higher (*P* <0.05; t-test) in the 0.3 m treatment than in the other two treatments from weeks one to four. The cuttings in treatments 1.2 m and 2.1 m did not start callusing until week two, when the two treatments did not differ significantly (*P* >0.05; t-test) from each other in this variable. This difference was maintained throughout the four weeks of the experiment. There were no significant differences between cuttings harvested from heights 1.2 and 2.1 m in terms of callus formation throughout the four weeks of the experiment, but values from the 0.3 m treatment were significantly higher (Figure 6.1b).

Cutting mortality

Although 2% of the cuttings from the 0.3 m treatment had died by the end of week one, the rate of cutting mortality in this treatment remained lower than in the other treatments from week two onwards. At weeks three and four, values in this treatment were significantly lower than in the other two treatments (*P* <0.05; t-test), which did not differ significantly (*P* >0.05; t-test) from each other (Figure 6.1c).

Leaf shedding

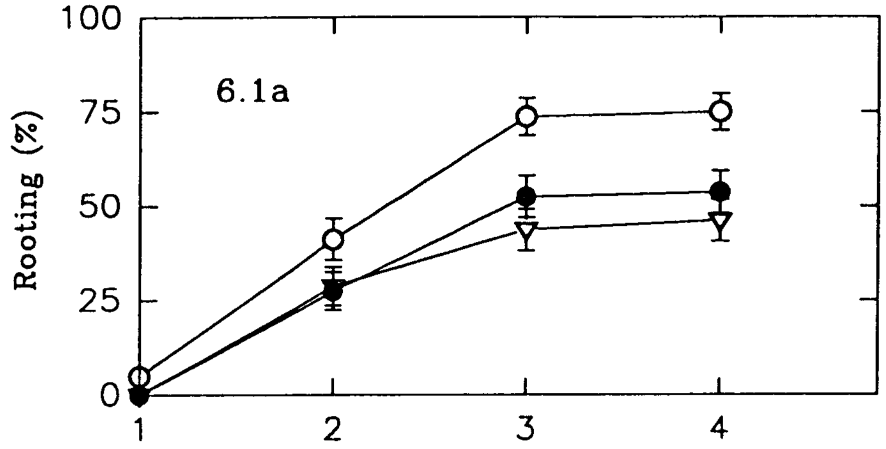
The cuttings obtained from 0.3 m stump height started shedding their leaves in week one, although significantly fewer cuttings shed their leaves in weeks two, three and four than those from stump heights of 1.2 and 2.1 m (*P* <0.05; t-test). However, there was no significant difference (*P* >0.05; t-test) between the two stump heights (1.2 and 2.1 m) in terms of percentage leaf shedding by cuttings throughout the rooting period.

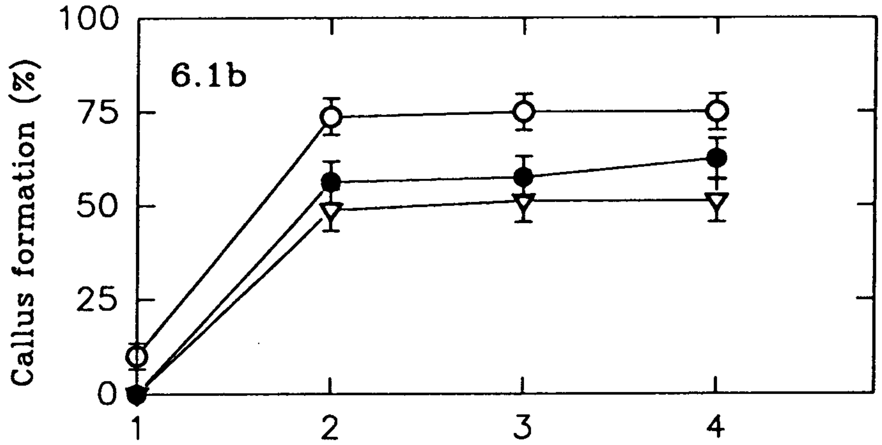
Shoot formation

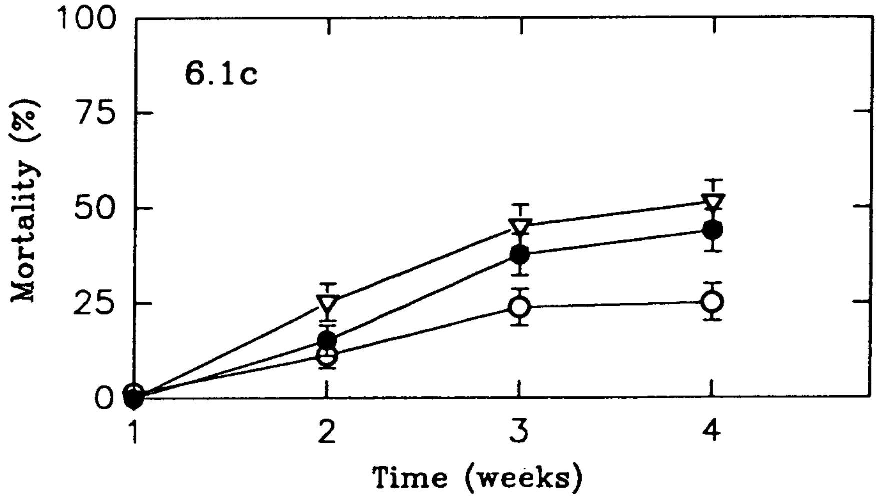
By the end of week one, 2% of the cuttings had formed new shoots in height treatment 1.2 m, although the proportion of cuttings with new shoots in this treatment was subsequently relatively low. Percentage shoot formation in the 0.3 m treatment was significantly higher than the other two treatments (P <0.05; t-test) at weeks three and four, with 51% of the cuttings having shoots by week four (Figure 6.1e). However, treatment 1.2 m did not differ significantly (*P* >0.05; t-test) in new shoot formation percentage from treatment 2.1 m

Number of roots per cutting

Mean root number per rooted cutting tended to decline with increasing stump height (Figure 6.1f). The highest mean number of roots (8.2) per cutting was associated with treatment 0.3m. At the final assessment at week four, treatment 0.3 m was significantly higher (P < 0.05; t-test) than treatment 2.1 m in this variable. However, there were no significant differences between treatments 0.3 m and 1.2 m and between 1.2 m and 2.1 m in terms of mean root number er rooted cutting (P > 0.05; t-test).


6.1a
Rooting (%)
100
75
50
25
0
1
2
3
4


6.1b
Callus formation (%)
100
75
50
25
0
1
2
3
4


6.1c
Mortality (%)
100
75
50
25
0
1
2
3
4
Time (weeks)

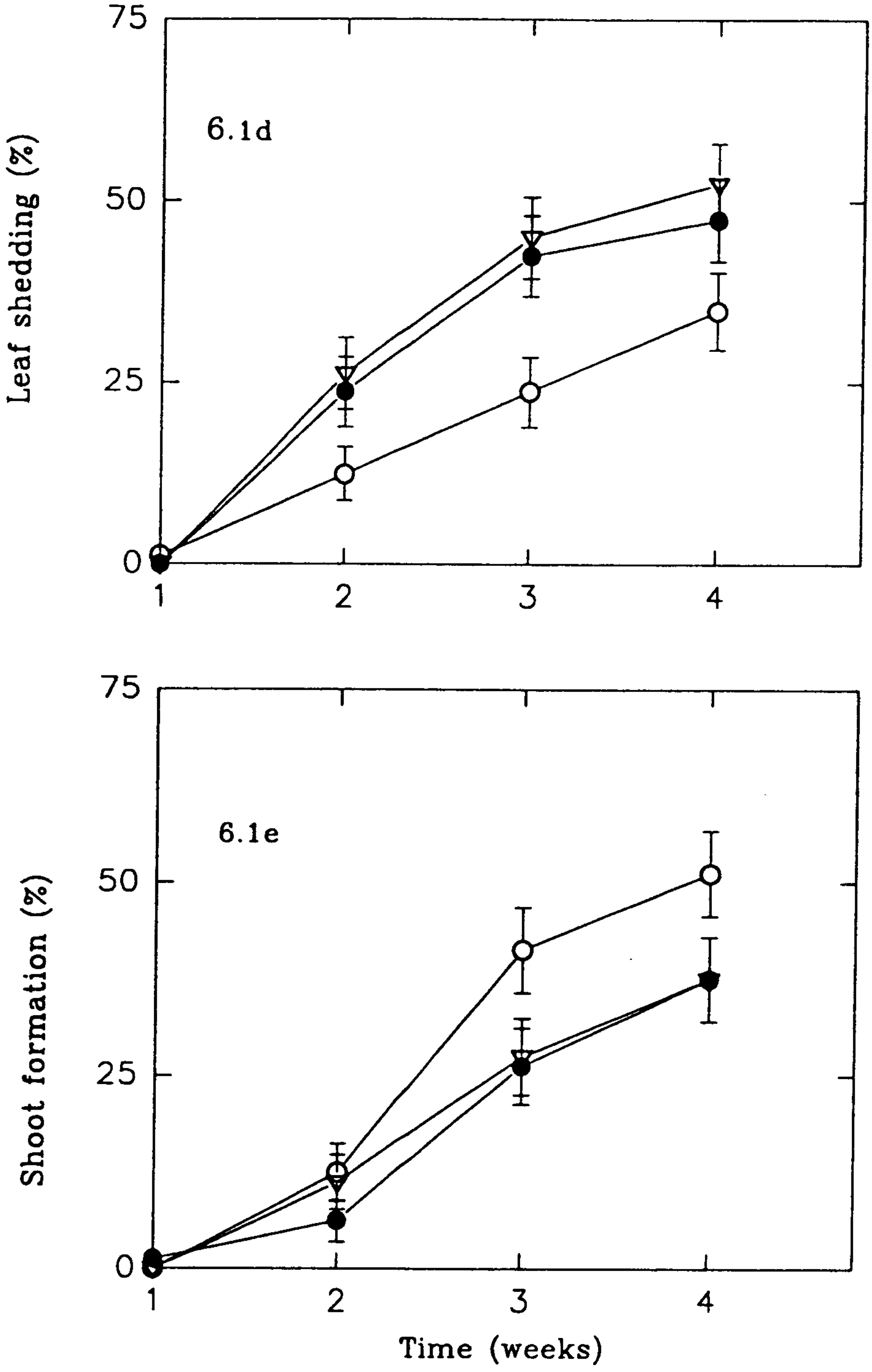


Figure 6.1: Effect of 3 stump heights on rooting of leafy stem cuttings of *R. heudelotii*, (○ = 0.3 m, ● = 1.2 m and ▽ = 2.1 m). Bar = ± SE. (n = 60).

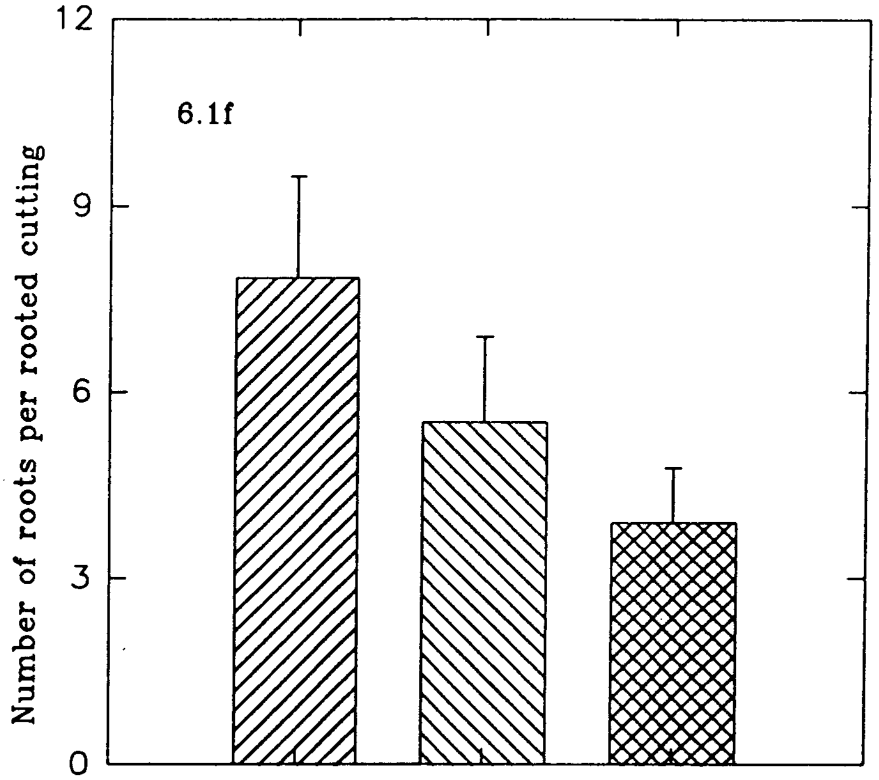


Figure 6.1f: Effect of 3 stump heights on number of roots per rooted cuttings of *R. heudelotii*. ▨ = 0.3 m, ▧ = 1.3 m and ▩ = 2.1 m) (after 4 weeks). Bar = + SE. (n=60).

6.3 Experiment 2: Effect of stockplant light environment on rooting of leafy stem cuttings of *Ricinodendron heudelotii.*

6.3.1 Introduction

One of the major factors in the stockplant growth environment which influences the rooting of cuttings is irradiance, as discussed in chapter one. Many studies have been carried out in recent years to investigate the effects of light environment on stockplant growth and rooting ability, including studies on tropical hardwood species such as *Triplochiton scleroxylon* (Leakey and Storeton-West, 1992), *Khaya ivorensis* and *Lovoa trichilioides* (Tchoundjeu, 1989), *Cordia alliodora* and *Albizia guachapele* (Mesén, 1993). Results of these studies have generally indicated that low irradiance and low red:far red ratios are associated with higher rooting percentages, although each species has its own optimum light requirements (Leakey and Storeton-West, 1992). For example, Leakey and Storeton-West (1992) demonstrated that at 1.6 and 6.3 R:FR ratios, and irradiances of 106 and 249 μmol m^{-2} s^{-1}, rooting percentages of *Triplochiton scleroxylon* cuttings were higher (50% and 80%) at a R:FR ratio of 1.6 and an irradiance of 106 μmol m^{-2} s^{-1} than at higher values. Such studies have not previously been conducted with *Ricinodendron heudelotii.* The present experiment was carried with the principal objective of finding out the effect of shading stockplants on rooting ability of cuttings taken from them.

6.3.2 Materials and Methods

For this experiment, two trees of the same clone were selected randomly from each of four clones not randomly selected because the number of stockplants per clone was limiting. Consequently, only clones with more than two stockplants were used in this experiment and also in experiment three on fertilizer application. Two treatments were randomly assigned to the two selected stockplants. The treatments were "shaded and non-shaded" with light filter (Strandfilter E1 22Cx417, No. 417

Steel Blue and No. 444 Azure Blue, Northern Light , Assembly street Leith, Edinburgh, UK), resulting to a paired plot experimental design where the probability of one of the clonal stockplants being assigned any of the two treatments was 0.5. They were cut to a height of 0.6m on the 26th April 1993. A square frame of wooden poles was pinned around one of the two stumps of each of the four clones in such a way that the stump was half a metre from the frame. Four equal pieces of wood were used to make a top for covering with a piece of light filter. The light filter was cut to size and nailed on the wooden poles at the corners to completely enclose the stump (Plate 6.3). This covering was completed two days after stumping the plants. Both the enclosed and non-enclosed stumps sprouted equally well. The number of shoots per stump ranged from five to eight.

During the period when the stumps sprouted, light measurements had to be taken inside the light filter space at the top of the stockplant and outside above the light filter. To this effect, two quantum sensors (Skye Instruments Ltd., Llandrindod Wells, UK) were used to measure the irradiance and the R:FR ratios of the shielded and unshielded stockplants. The light data are presented in Table 6.1.

Table 6.1: Light data of the shaded and the unshaded stockplants in μmol m^{-2} s^{-1}.

Clone	Range of irradiance		Mean irradiance in μmol m^{-2} s^{-1}	
Shaded	Outside	Inside	Outside	Inside
CN 2006	110-160	41-51	126 ± 13	46 ± 5
CN 2032	120-181	21-51	156 ± 25	34 ± 9
CN Al1	111-180	21-50	176 ± 19	34 ± 8
CN AL3	111-160	21-61	133 ± 18	43 ± 12
Shaded and unshaded stockplant mean irradiance			138 ± 13	40 ± 6

Plate 6.3: Stockplants shaded with light filter to study the influence of reduced irradiance on the rooting ability of the cuttings of *Ricinodendron heudelotii* taken from them.

On the 2nd of June 1993, one shoot was obtained from each of the eight plants of the four clones, treated as described in experiment ten to yield four blocks of one hundred and sixty cuttings in the same propagator. The cuttings were assessed as described above.

The shoots grown in the shade were feeble and fragile to harvest and handle. They had longer internodes, looked chlorotic, with thinner leaves and did not look as dark green as those in the open. They had a greater portion of softwood than those from the stockplants grown in the open. The leaves were free from insect attack and did not look as broad as those in the open field.

Analysis

Analysis of variance using SAS (1980) was carried out to determine the effect of reduced stockplant irradiance on the subsequent rooting ability of the cuttings of *R. heudelotii*, followed by Fisher's t test (LSD) for comparing differences between many means. Data of percentage rooting was transformed by the formula arcsin $\sqrt{\%}$ prior to analysis. Standard errors and confidence limits were calculated by the procedure of Snedecor and Cochran (1980) for binomial data.

Analysis of deviance by stepwise regression in Genstat 5 (Payne *et al.*, 1987) was utilized to determine the influence of treatment, node position, clone, stem length, block and stem diameter at the cutting base on the rooting ability of the cuttings of *R. heudelotii*.

6.3.3 Results

Rooting percentage

The time of rooting was the same in the two treatments. Cuttings from both shielded and unshielded stumps commenced rooting in the second week when 45% of those from the latter treatment had produced roots, while only 23% of those from the former had rooted. In terms of percentage rooting, cuttings from stumps in full light were significantly higher than those from screened stumps (P <0.05; t-test) by the third week of the assessment period. At the end of the experiment in week four, analysis of variance test revealed that there were no significant differences (P >0.05) between the two treatments in this trait (Table A21). However, confidence limits and analysis deviance (Table 22) showed that there were significant differences between these two treatments (P < 0.001). Generally, rooting percentage increased between weeks one and three. By week four, 86.3% and 61.3% of the cuttings from stumps without light filter and those with filter had rooted respectively (Figure 6.2a).

Analysis of deviance by stepwise regression, conducted at the end of the experiment in week four, showed that while the rooting ability of *R. heudelotii* was significantly affected by the reduced light on the stockplants from where the cuttings were harvested and by experimental blocks, it was not significantly affected by clone and cutting diameter at the base (Table A22).

Callus formation

Cuttings did not differ significantly in callusing between both stockplant treatments (with and without light filter), , although the proportion of callused cuttings tended to increase from weeks one to four, when over 85% of them had callused (Figure 6.2b). In this experiment, cuttings callused irrespective of whether or not the stockplants from where they were harvested received any light cover pre-treatment.

Cutting mortality

By the end of the second week, no cutting from either screened or unscreened stockplants had died. However, between weeks two and four, percentage mortality increased, reaching values of 15 and 20% by the fourth week in the two treatments respectively. Although cuttings taken from stumps in full light displayed higher values of variable than those take from treated stumps, there were no significant differences between the two treatments (*P* <0.05; t-test) (Figure 6.2c).

Leaf shedding

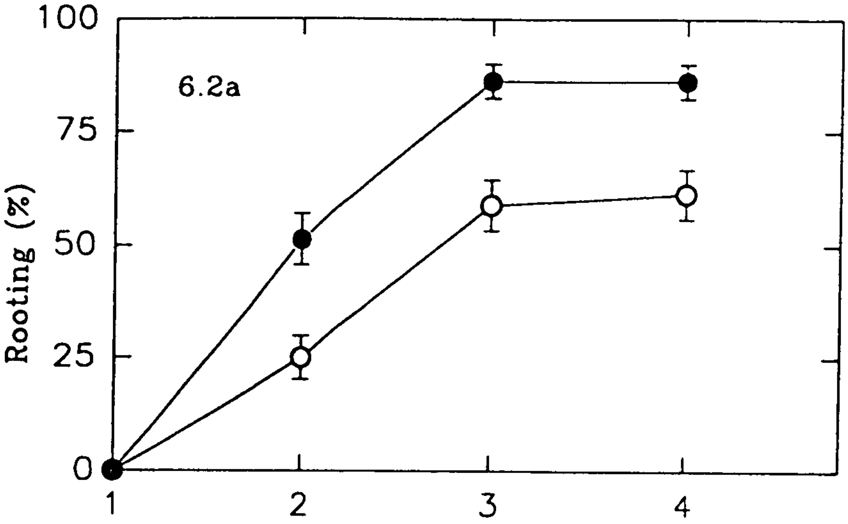
The cuttings from the treated and untreated stockplants started shedding their leaves in the second week, when the proportion of cuttings which shed their leaves was significantly (*P* <0.05; t-test) higher in shaded stumps than those which received full irradiance. This difference between the two treatments was maintained throughout the four weeks of the experiment, while the percentage leaf shedding in both treatments tended to increase progressively until the fourth week (Figure 6.2d).

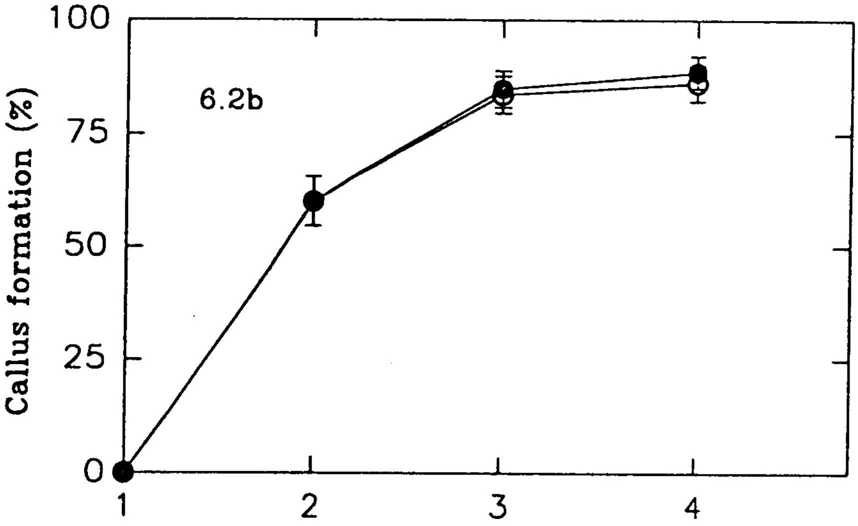
Shoot formation

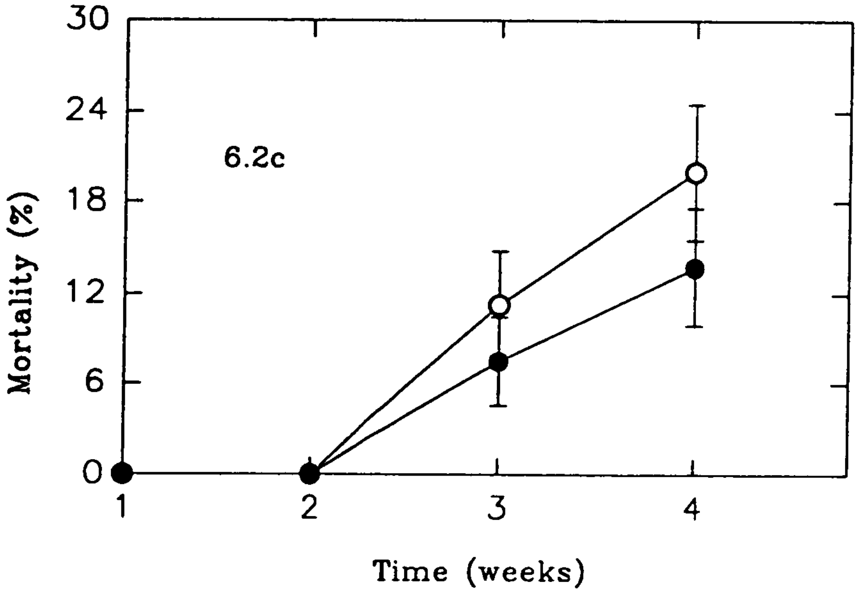
Cuttings from the stockplants in full light had a significantly higher number of new shoots by week two than those from shielded stockplants (*P* <0.05; t-test). The percentage of new shoot formation in the two treatments tended to increase between weeks one and four (Figure 6.2e), while maintaining the difference between treatments.

Number of roots per cutting

A higher mean root number (6) was recorded in the cuttings that received the full light treatment before harvest than in those shaded with the light filter, after a rooting period of four weeks (*P* <0.05; t-test) (Figure 6.2f).


6.2a
Rooting (%)
0
25
50
75
100
1
2
3
4


6.2b
Callus formation (%)
0
25
50
75
100
1
2
3
4


6.2c
Mortality (%)
0
6
12
18
24
30
1
2
3
4
Time (weeks)

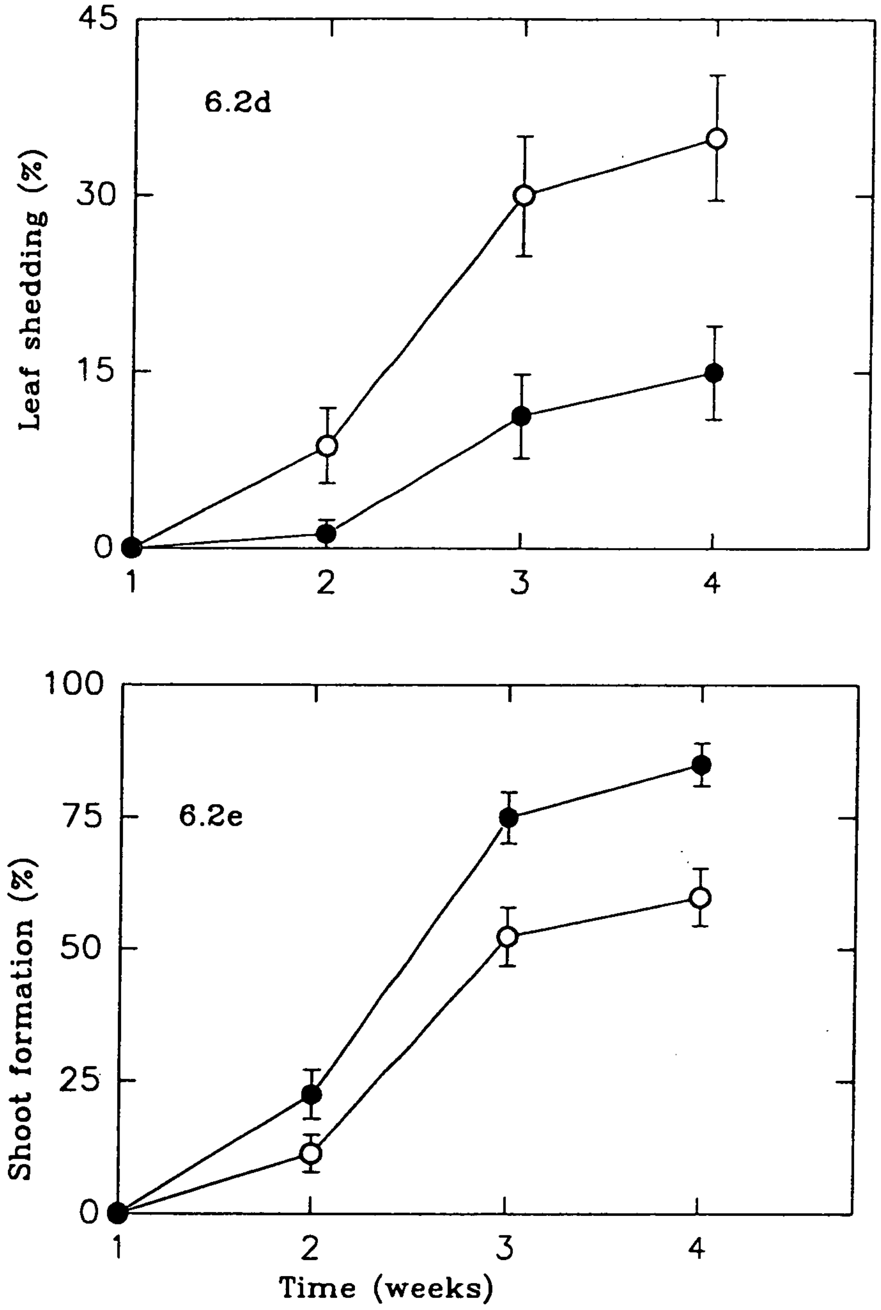


Figure 6.2: Effect of light filter on rooting of leafy stem cuttings of *R. heudelotii*, (○ = with filter and ● = without filter). Bar = ± SE. (n = 80).

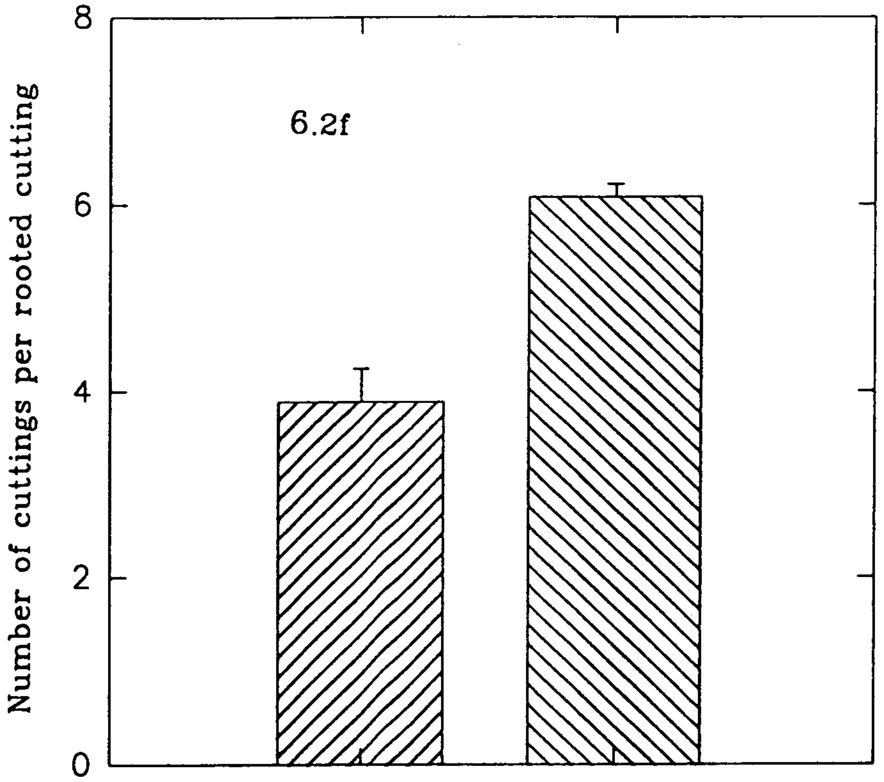


Figure 6.2f: Effect of filter on number of roots per rooted cuttings of *R. heudelotii*. (▨ = +filter and ▧ = – filter) (after 4 weeks). Bar = + SE. (n = 80).

6.4 Experiment 3: Effect of fertilizer (NPK) application on rooting of leafy stem cuttings of *Ricinodendron heudelotii*

6.4.1 Introduction

Recent studies carried out to investigate the influence of NPK fertilizers on stockplant growth and rooting ability have demonstrated the role of applied nutrients in stimulating root formation in the cuttings of some tropical timber species (Tchoundjeu, 1989; Leakey, 1983; Leakey and Storeton-West, 1992). It was found, for example, that the highest rooting percentages in cuttings of *Triplochiton scleroxylon* occurred when fertilizers were added (Leakey, 1983), and that 50 g of NPK fertilizers added to each stockplant, enhanced the rooting of the cuttings of *Lovoa trichilioides* (Tchoundjeu, 1989). However, it was found that unrestricted application tended to be harmful to cuttings of *Khaya ivorensis* stockplants, which were treated with increasing amounts of NPK fertilizers (Tchoundjeu, 1989). The harmful effects of a relatively high dose (150 g per plant) of NPK fertilizer (mainly rotting of roots) was attributed to the high content of reducing sugar and the low content of starch in the leaves during rooting (Leakey *et al.*, 1992). The fertilizer requirements for maximum rooting of stockplants of most tropical tree species are not known, although requirements clearly vary from species to species (Leakey *et al.*, 1992). This experiment was designed to study the effect of application of NPK to stockplants on rooting of leafy stem cuttings of *Ricinodendron heudelotii*, with the aim of defining appropriate stockplant management protocols for this species.

6.4.2 Materials and methods

Some clonal stockplants raised in earlier experiments were planted out near the propagation unit in the premises of the Kumba Forestry Research Station in May 1992 (like those in Plate 6.1). Two stockplants were selected from each of nine clones. One plant of each pair was randomly selected and labelled for fertilizer or non-fertilizer treatments. There were, therefore, nine stockplants that received fertilizer and nine that received no fertilizer from nine clones in a paired plot experimental design. In mid December 1992, trenches were dug round each plant at a radius of 50 cm. All the eighteen stockplants were watered twice a day to field capacity until mid-March 1993 when the rains were heavy enough to keep the plants well watered. Fifty grams of fertilizer (NPK 20:10:10) granules were applied to the nine selected stockplants, by placing the granules in the trenches three times a month until mid-July 1993. During this period, weeding the plants and spraying them with a systemic insecticide were undertaken when necessary. Ten stockplants of five clones, which were stumped at 75 cm above the ground were finally utilized in the rooting experiment. The stumps produced a minimum of four and a maximum of thirteen shoots per stump. By mid-August, the shoots were large enough to enable at least five cuttings to be taken from each.

On August 18th, four sets of ten shoots were collected from each of the ten stockplants treated as stated above (five fertilized and five not fertilized). Five cuttings were taken from each shoot of each set (10 shoots per set x 5 cuttings per shoot x 4 sets) were treated with 40 μg of IBA with the leaves trimmed to 50 cm^2 and set into sawdust according to node position in one of ten lines in one of four blocks, after drying off the cutting base in an air current from a fan. Blocking was done according to the number of shoots per stockplant, resulting in four blocks each containing five clones with a pair of treatments per clone. The cuttings were assessed weekly for four weeks as described in earlier experiments.

Analysis

Analysis of variance and that of deviance were applied to assess the effect of fertilizer (NPK) application to the stockplant on the subsequent rooting ability of the cuttings of *R. heudelotii* and other parameters of the cuttings assessed in this experiment at the end of the experiment in week four as described in earlier experiments.

6.4.3 Results

Rooting percentage

The rate of rooting was similar in the cuttings obtained from the fertilized and control stockplants. By week two, rooting had commenced in the two treatments, and the percentage of rooted cuttings was significantly higher in the cuttings from the fertilized plants than in those from the controls (75% versus 50% respectively). The significant difference between the two treatments ($P < 0.05$; t-test) was maintained through weeks three and four (Figure 6.3a). At the end of week four, analysis of variance test confirmed that there were significant differences ($P < 0.05$) in the percentage of rooted cuttings between the two treatments (88.0% in cuttings from fertilized stockplants versus 67.0% from the control) (Table A23).

When the data was analyzed by stepwise regression, it was found that the rooting ability of *R. heudelotii* in this experiment was significantly affected by fertilizer application and by stem diameter at the cutting base, but was not significantly affected by block, clone and stem length (Table A24).

Callus formation

No cutting in either treatment had callused by the end of week one, but percentage callus formation increased rapidly, such that 62.3% of cuttings had callused in both treatments by week two. From week two to week four, there was a significant difference ($P < 0.05$; t-test) between fertilized and non-fertilized cuttings, in terms of percentage callus formation, the fertilized treatment being consistently higher (Figure 6.3b). By week four, the difference between the two treatment means was much smaller than at weeks two and three.

Cutting mortality

By week two, the proportion of dead cuttings in the control treatment and was significantly higher (*P* <0.05; t-test) than in the fertilized treatment. Mean cutting mortality increased markedly between weeks two and three in the non-fertilized treatment (Figure 6.3c), such that by week four, 35% of the cuttings in the non-fertilized treatment had died, while only 13% of the cuttings in the fertilized treatment were dead.

Leaf shedding

Cuttings from the fertilized stockplants did not shed any leaves until the second week after insertion, while cuttings from the control stumps had shed more than 1.5% of their leaves by the end of week one. From the second week, the percentage of cuttings that shed their leaves increased steeply in both treatments. By the fourth week, there was no significant difference (P >0.05; t-test) between the two treatments, although the control treatment was significantly higher in weeks two and three (Figure 6.3d).

Shoot formation

The percentage of new shoot formation in the cuttings of both treatments was similar in the first two weeks, although significant differences occurred in weeks three and four, resulting from a much higher proportion of cuttings forming new shoots in the fertilized treatment than in the control (Figure 6.3e).

Number of roots per cutting

At the end of the four week rooting period, cuttings from fertilized stockplants had a higher number of roots per rooted cutting than (*P* <0.05; t-test) those from stockplants without fertilizer (Figure 6.3f).

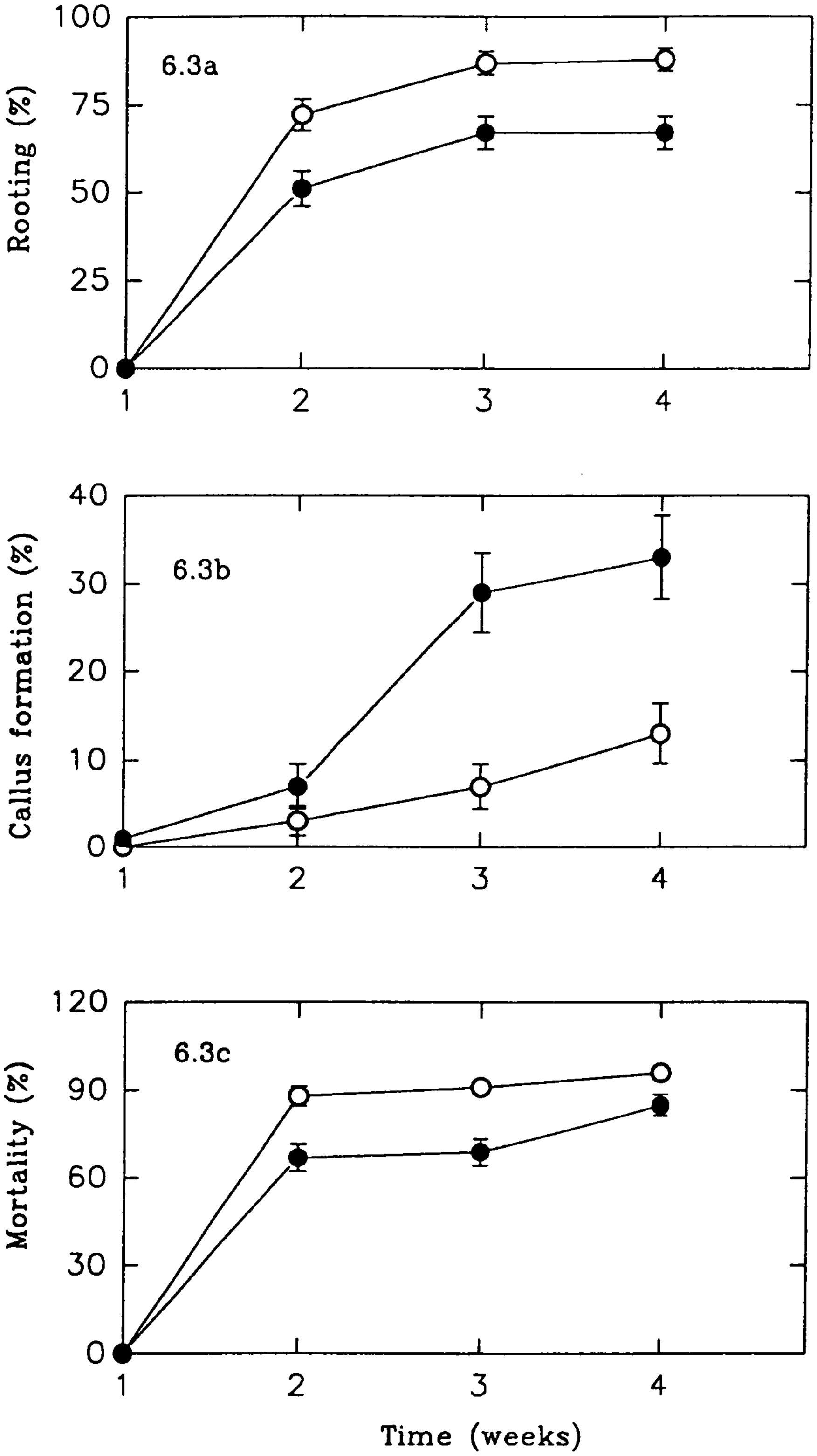
6.5 Discussion

Rooting percentage

Studies have shown that plant propagation by rooting leafy stem cuttings (among other factors) depends on the physiological state of the stockplants (pre-severance factors) from where the cuttings are obtained (Andersen, 1986; Moe and Andersen, 1988, Hartmann and Kester, 1983). Consequently, stockplant management procedures aimed at the continuous and prolific production of cuttings are being introduced increasingly for fruit and timber trees (Leakey, 1983). Some of these procedures, which have been applied successfully to tropical stockplants, include stumping (hedging, pruning and stooling), fertilizer application, shading (to control light and temperature) and watering (Tchoundjeu, 1989; Leakey, 1983; Hartmann and Kester, 1983).

The three experiments in this chapter investigated the effect of different stump heights, shading and fertilizer application to stockplants of *R. heudelotii* on the subsequent rooting ability of cuttings obtained from them.

The rooting results of these three experiments showed that these factors constitute some of the major requirements in the stockplant management of this species for sustained supply of easy-rooting cuttings. The highest percentage of rooted cuttings recorded in experiments 1, 2 and 3 were 75%, 86% and 88% respectively. The percentage rooting results in the fertilizer experiment were higher than those in the stump height and light treatments. This may indicate that plant nutrition is a more fundamental requirement by stockplants for subsequent rooting of cuttings obtained from them than light and different stump heights, since it has been shown that cuttings from etiolated stockplants and from unstumped stockplants have been rooted


6.3a
Rooting (%)
100
75
50
25
0
1
2
3
4
6.3b
Callus formation (%)
40
30
20
10
0
1
2
3
4
6.3c
Mortality (%)
120
90
60
30
0
1
2
3
4
Time (weeks)

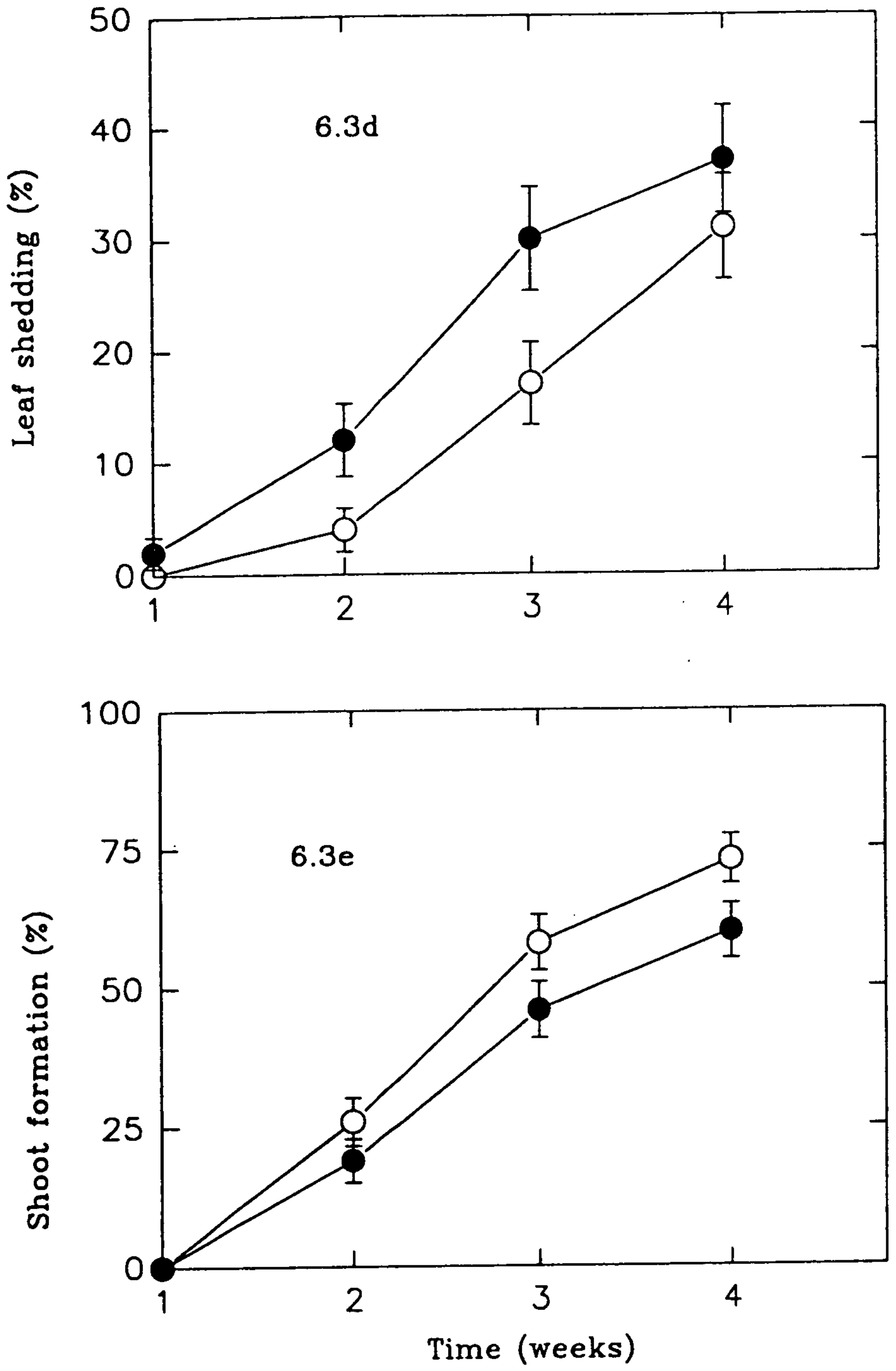


Figure 6.3: Effect of fertilizzer application on rooting of leafy stem cuttings of *R. heudelotii*, (○ = + fertilizer and ● = - fertilizer). Bar = ± SE. (n = 100).

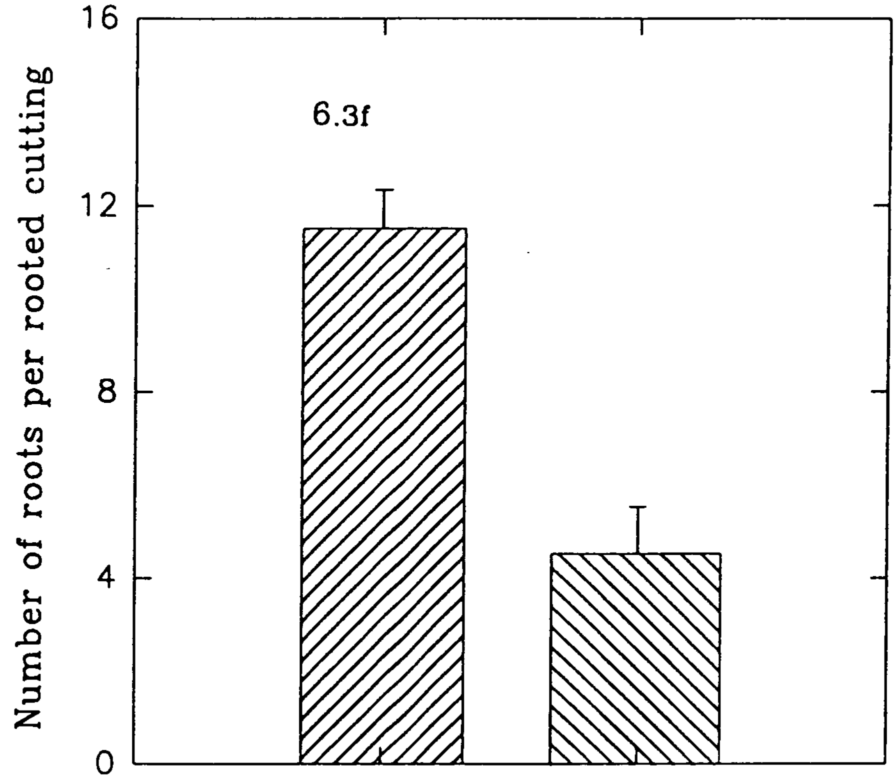


Figure 6.3f: Effect of fertilizer on number of roots per rooted cuttings of *R. heudelotii*.. ▨ = + fertilizer and ▧ = − fertilizer) (after 4 weeks). Bar = + SE. (n = 100).

successfully (Bonga, 1981; Raviv, 1981; Maynard and Bassuk, 1988). In general terms, however, all the three factors form part of the environmental requirements for successful stockplant management (Leakey *et al.*, 1994).

In the experiment on different stump heights, percentage rooting tended to decrease with increasing stump heights, with the highest percentage rooting of 75% associated with the 0.3 metre treatment and the lowest of 46% was recorded in the 2.1 m treatment. This concurs with work on ***Triplochiton scleroxylon*** cut to different heights of 0.5 m, 1.0 m, 1.5 m and 2.0 m, where the highest percentage of rooted cuttings associated with 0.5 m treatment was 58% and that recorded in the 2 m stump height treatment was 30% (Nketiah, 1994). Leakey (1983) also reported a similar percentage rooting pattern observed on the cuttings obtained from stockplants of the same species cut to different heights up to one metre. However, these stockplants were managed under glasshouse conditions in the temperate zone, while the results reported by this author and Nketiah (1994) were obtained in the tropics from stockplants planted out in the field. In studies involving the pruning of hedges of Sitka spruce, Morgan and Mason (1992) found that the rooting ability of cuttings declined with increasing heights of the hedges.

The rooting percentage ranged from 46.3 to 75% in cuttings from the tallest and lowest stockplants, suggesting that rooting could improve considerably if the other factors in the stockplant environment were optimized. These results are consistent with the observation of Leakey (1983) that the decrease in the rooting ability of the cuttings of *T. scleroxylon* with increasing height of the stockplants appeared to be related to competition between the shoots (Figure 7.1g). The rooting decrease with stump heights might have also been due to the different positions of the shoots from which cuttings were taken in agreement with Borchert (1978), who indicated that juvenility appears to be related to the distance of the parts of the plant from the roots of the plant. The juvenility of the shoots from which cuttings are taken for rooting on a stockplant has in turn been shown to decrease with distance away from the base and the axis of the stockplant (Wiltshire and Reid, 1992; Borchert, 1976; Bonga, 1981). It has been reported that the cambium near the base of the stockplant trunk is more juvenile than that in the upper part of the trunk, and may retain some juvenile characteristics for many years (Bonga, 1982). The decline in the rooting ability of *R. heudelotii* cuttings with stump height could have partly been attributed to the cambial activities of its trunk and the shoots from which the cuttings were taken in accordance with this hypothesis. Longer distances between the root and the shoot system of stockplants from the base up to the crown may pose a transport problem of nutrients, water, growth regulators, reserves and current carbohydrates arising from shoots along the trunk. These growth requirements may tend to decrease with distance away from the root and base, thereby forming a relative concentration gradient of these growth substances at the base to the detriment of shoots located at greater distances further up the crown of the stockplant (Borchert, 1976; Fortanier and Jonkers, 1976). This could account for the decline in the rooting pattern displayed by the cuttings of *R. heudelotii* in the present study with increasing stump height.

Interestingly, however, more than 46% of the cuttings from the 2.1 metre stump height rooted within four weeks, although the growth performance of the plants from cuttings at such heights in terms of height and fruiting time still remains to be seen in the field. This is important since the products of major interest to meet man's needs are the fruits. In addition to this, it is uncertain whether or not these stockplants were males or females as they were stumped at the sapling stage of their growth, when they could not display the phenotype of interest, in accordance with the observation of Bonga (1981; 1982;), Brix (1973) and Biondi and Thorpe (1981) that mature trees must have expressed their genetic potential or past performance throughout the rotation before selection for propagation. However, the results have demonstrated that rooting of cuttings is possible at that height, or even at higher points on the stumps.

In the experiment on reduced irradiance on stockplants, the percentage of rooted cuttings differed significantly between cuttings from the shielded and unshielded stockplants, although ANOVA test showed no significant differences between these two treatments (Table A21). The value of those from the unscreened stockplants was much higher than that of the others (86% versus 61%). These results are not in agreement with those of Mesén (1994), who did not find any significant difference between the percentage of rooted cuttings from shaded stockplants and that of cuttings from stockplants of *Cordia alliodora* in direct sunlight. However, they contradict the results of Nketiah (1994), who found that in a shading experiment, high percentage of rooted cuttings was associated with shaded stockplants of *T.scleroxylon* and cuttings with a low rooting percentage were taken from stockplants in direct sunlight. This could be attributed to the differences in irradiance of 50% received in the shaded *T. scleroxylon* stockplants as against the irradiance of 28.6% received in the shaded stockplants of *R. heudelotii* in this study. The results are also in contrast with the view that the rooting ability of cuttings is higher at lower than at higher irradiance as pointed out by Moe and Andersen (1988) and Andersen (1986). The mean irradiance within the screened stockplants was 39.4 ± 9.9 μmol m^{-2} s^{-1} and that on the stockplants in direct sunlight, was 135.8 ± 29.43 μmol m^{-2} s^{-1} during the rainy season. The lower percentage of rooted cuttings from stockplants within the screen may be attributed to the low irradiance as compared to the high values recorded on the control stockplants. Once again, this is contrary to Leakey and Storeton-West (1992) who recorded the highest percentage of rooted cuttings from the stockplants of *Triplochiton scleroxylon* grown under low irradiance of 106 μmol m^{-2} s^{-1} and R:FR ratio of 1.6, and the lowest rooting results at high irradiance and R:FR ratio of 246 μmol m^{-2} s^{-1} and 6.3 respectively, although this was obtained from artificial source.

Although the reason for associating enhanced rooting of cuttings with reduced irradiance given to the stockplants from which they are taken is not clear (Hansen, 1976;), it could be ascribed to the fact that levels of certain natural growth inhibitors, which may reduce rooting, are higher in plant tissue grown in light (Eliasson, 1971; Tilburg, 1974). Tilburg (1974) also suggested that high content of native auxins in the growing points of plants grown in high light, does not get to the basal rooting zone of cuttings, leaving this zone low in auxins. High irradiance may cause the production of carbohydrate above optimum level, which would not interact efficiently with auxin in root formation (Nanda *et al.*, 1971; Leakey and Storeton-West 1992). However, higher irradiances have been found to increase auxin transport and accumulation of auxin at the base of pea plants (Baadsmand and Andersen, 1984). For example, applying the immunoassay technique to ***Chrysanthemum***, the data obtained on the cuttings of this species indicated more auxin to be present in the plants grown under 40 W m^{-2} than under 4 W m^{-2} (Weigel *et al.*, 1984). In the present study, the stockplants in direct sunlight, at that period of the rainy season with mean irradiance of 138.1 μmol m^{-2} s^{-1}, might have received a level of irradiance very close to optimum for this species, since the shaded stockplants which received 28.6% (39.5 μmol m^{-2} s^{-1}) of it produced cuttings which registered a lower rooting percentage.

In the third experiment, on stockplant nutrition, it was found that fertilizer (NPK) application greatly enhanced the rooting ability of *R. heudelotii*. The percentage of rooted cuttings from fertilized stockplants was significantly (P <0.05) higher than that of cuttings from the control. In a similar experiment conducted on *T. scleroxylon*, Nketiah (1994) found that the proportion of rooted cuttings from fertilized stockplants was significantly higher than that of cuttings from stockplants without fertilizer. These results are in agreement with Leakey (1983), who found with the same species that the percentage of rooted cuttings was significantly higher, particularly in basal shoots, in cuttings from stockplants of the same species gives a higher concentration of fertilizer (4.0%) than in those from treated stockplants with a lower concentration (0.4%). Similar results were obtained by Tchoundjeu (1989) who found that NPK application to the stockplants of *L. trichilioides* considerably enhanced the rooting ability of the cuttings taken from them. In a separate experiment with the same species, Leakey and Storeton-West (1992) also found that the application of liquid NPK improved rooting in cuttings taken from stockplants grown at high irradiance from 9.3 ± 3.1% to 28.7 ± 4.8%, but irradiance without fertilizer had effect on rooting. Cuttings from geranium (***Pelargonium hortorum***) stockplants fertilized with NPK were also found to have higher rooting percentages at low and medium levels of N, but were not affected by P and K (Haun and Cornell, 1951, cited by Hartmann and Kester, 1983). However, Mesén (1994), found that fertilization (NPK) application significantly reduced the rooting percentage of the cuttings of *Cordia alliodora*. This suggests that the fertilizer concentration was too high for *C. alliodora* and tended to enhance a production of starch and carbohydrate in the stockplant of this species, which might have inhibited photosynthesis resulting in end-product inhibition as reported by Leakey and Storeton-West (1992), with an adverse effect on rooting.

The influence of fertilizer (NPK) application to stockplants on the rooting ability of cuttings harvested from them may be ascribed to the enzymatic reduction of nitrogen to nucleic acid, amino acids and proteins, which play active roles in rooting and development of other growing points of cuttings during propagation (Hartmann and Kester, 1983; Blazich, 1988). NPK fertilizers are also known to enhance the production of chlorophyll in leaves and plant proteins such as ferrodozin and ribulose diphospate carboxylase (Salisbury and Ross, 1978), which are all involved in photosynthesis for the production of current assimilates needed for rooting (Leakey and Coutts, 1989; Newton *et al.*, 1992b). This might have accounted for the rooting results of the present experiment.

Callus formation

The rate of callus formation was higher in the stump height experiment than it was in the light and fertilizer experiments, commencing by the end of the first week after cutting insertion. The rate of callus formation was similar in the latter two experiments, starting in week two in each case. However, the percentage of callused cuttings varied in the fertilization experiment much more than it did in the light experiment. While the percentage of callused cuttings in the fertilized and control treatments of experiment 3 was 67% and 88% respectively, that in the screen and control treatments of experiment 2 was the same (60%) each (Figure 6.1b, 6.2b, 6.3b). The percentage of callused cuttings increased in the three experiments with time to the end in week four after insertion, when the highest values of 75%, 89% and 96% were associated with the stump height, light and fertilization experiments respectively. In all the three experiments, percentage callus formation was found to be positively related to percentage rooting, with the highest percentage of both traits being recorded in the same treatment in each experiment (Figure 6.1b, 6.2b, 6.3b).

Experiment 1 exhibited a decreasing trend with increasing stump heights. In this regard, percentage callused formation was consistent with the percentage of rooted cuttings which displayed a negative relationship with stump height.

Except in height treatment 1.2 m, all the cuttings which callused in this experiment also rooted. The fact that both traits were positively related to height treatments indicates that rooting is probably dependent on callus in this experiment. This is in contrast with the view of Hartmann and Kester (1983) that callus formation is independent of root production during the propagation of some plant species.

In experiment 2, percentage callus formation did not commence until week two, when the same proportion (60%) of cuttings in each of the two treatments callused. From week three to the end of the experiment in the fourth week, there was very slight variation in the percentage of callused cuttings in the two treatments, such that 89% and 86% were recorded in cuttings from the shielded stockplants and in those from the control respectively. The percentage of callused and that of rooted cuttings tended to increase in the control treatment. However, out of the 88% of the cuttings that callused, only 2% did not root, as against the 75% that callused and all rooted in the highest treatment (0.3 m) in experiment 1.

In the fertilizer experiment, the percentage of callused cuttings was higher among the cuttings from the fertilized stockplants than it was among those from the control treatment from the second to the last week of the experiment. However, the proportion of cuttings that callused was positively related to that which rooted from the same time, when callusing started to the end of the experiment in week four (Figure 6.3b). The results indicate that out of the 96% of the cuttings from the fertilized stockplants, which callused, only 88% successfully rooted and about 8% failed to root. While 85% of the cuttings from the unfertilized stockplants callused, only 67% of them rooted and 21% did not root. This demonstrates that nutrient reserves may be necessary to make root primordia from root initials and grow through callus and differentiate into roots, in agreement with the observation of Bonga and Durzan (1982) who implicated lack of sufficient nutrient reserves in the non-differentiation of the cells behind the mass of callus into roots.

Mortality

The time of cutting mortality was different in the three experiments of this chapter, starting in the first week in experiments 1 and 3, but no cutting died in the second experiment until week three (Figures 6.1c. 6.2c, 6.3c). Percentage cutting mortality was more varied in experiments 1 and 3 than it was in experiment 2, such that by the end of the experiments in week four, it ranged from 25% to 51%, 14% to 20% and 13% to 33% in experiments 1, 2 and 3 respectively. The highest cutting mortality among the three experiments was associated with the 2.1 m stump height experiment, which might have been due to the origin of the shoot from where the cuttings were taken within the stockplants (Leakey *et al.*, 1994).

In the experiment on different stump heights, 1% of the cuttings in the 0.3 m height treatment died one week after insertion. However, in the subsequent weeks up to the last week of the experiment, the other two height treatments had lost a higher proportion of cuttings per week than the 0.3 m height treatment, which had the lowest cutting mortality throughout the four weeks of the experiment. Percentage cutting mortality tended to increase with stump height, contrary to rooting and callusing percentages, which decreased with decreasing stump heights. These results show that cutting mortality was inversely related to rooting and callusing.

The high cutting mortality associated with the stump height of 2.1 m may be attributed to competition among the many shoots at that height at the time of harvesting the cuttings, such that harvested cuttings did not have sufficient nutrient reserves. Some shoots which were growing too vigorously might have had too much nitrogen which could tend to inhibit photosynthesis by producing excess ferrodozin (Salisbury and Ross, 1978).

Cutting mortality in the light filter experiment did not commence until the third week, when a higher proportion of dead cuttings was associated with screened stockplants than with control treatment. By the end of the experiment in week four, 14% of the cuttings from the control treatment were dead, and 20% of those from the screened stockplants had also died. The percentage of cutting mortality was negatively related to that of callused and rooted cuttings.

The fact that the cuttings from shaded stockplants at lower irradiance tended to die more and root less than those from unshaded stockplants at higher irradiance is contrary to the observation made on many other tropical species such as *Triplochiton scleroxylon*, which survived and rooted more successfully at lower irradiance of 106 μmol m^{-2} s^{-1} than at higher irradiance of 246 μmol m^{-2} s^{-1} (Leakey and Storeton-West, 1992).

In the fertilizer experiment, 1% of the cuttings from unfertilized stockplants had died by the end of week one after insertion. By week two, 7% and 3% of the cuttings from the unfertilized and fertilized stockplants had died respectively. At weeks three and four, the percentage of dead cuttings was significantly higher (P <0.05; t-test) among cuttings from the unfertilized stockplants than it was among cuttings from fertilized stockplants (Figure 6.3c). The percentage of cutting mortality inversely related to the percentage of callused and rooted cuttings.

Cutting mortality in this experiment could have been related to inadequate nutrient reserves in the cuttings at the time of severance, when compared with mortality results of the cuttings from fertilized stockplants. This is supported by the fact that 33% of the cuttings from unfertilized stockplants died by the end of the experiment as against only 13% which died among the cuttings from fertilized stockplants. This emphasizes the importance of stockplant fertilization, since it does not only enhance rooting of cuttings taken from them, it also reduces cutting mortality. Insufficient nutrient reserves in the cuttings from this source might have resulted to the formation of callus at the cutting base, which could not differentiate further into roots (Bonga and Durzan, 1982).

Leaf abscission

The rate at which leaves abscised in the three experiments of this chapter was similar in experiments 1 and 3, where leaf abscission started in the first week after insertion, while in experiment 2, leaf loss did not commence until week two. However, the percentage of cuttings that shed their leaves was significantly different (P <0.05; *t*-test) in the treatments of experiments 1 and 2, but there were no significant differences (P >0.05; t-test) between the treatments of experiment 3 in this variable (Figures 6.1d, 6.2d, 6.3d). The highest leaf abscission in experiments 1, 2 and 3 was 53%, 35% and 37% respectively. This indicates that the highest percentage of cuttings that shed their leaves was associated with the stump height experiment, suggesting that this aspect of stockplant managements needs more attention in *R. heudelotii* than the other two factors tested in this study.

The high percentage of leaf abscission associated with increasing stump height could be attributed to the adverse effect of competition among the shoots from where the cuttings were taken. It could also be due to the age of the leaves, particularly those on cuttings taken at the basal end of the shoots from which some of the nutrients might have been withdrawn for storage or for use in the actively growing terminal and other buds.

High leaf abscission in the cuttings taken from the shielded stockplants might have been due to the fact that the leaf tissue did not develop enough chlorophyll to enable the leaves carry out photosynthesis, and this could lead to leaf shedding. The reduced activities of some leaves such as auxin, co-auxin, amino acid, enzyme and co-enzyme production could be so adversely affected by low irradiance that they may drop off.

Since leaf abscission was not significantly affected by fertilizer application to the stockplants, leaf shedding in this experiment could be attributed to cutting age, bacterial and fungal attack and water stress.

Shoot formation

The time when new shoots were produced by cuttings was different in the three experiments of this chapter. While 1.3% of the cuttings in experiment 1 had produced new shoots by week one, new shoot formation did not start in experiments 2 and 3 until week two. The treatments of experiment 2 exhibited significant differences in new shoot formation from week two throughout the experiment, while those of experiments 1 and 3 displayed significant differences in shoot formation from week three to the end of the experiments in week four (Figures 6.1e, 6.2e, 6.3e). The highest proportion of cuttings that produced shoots in experiments 1, 2 and 3 was 51%, 85% and 73% respectively. This shows that the highest percentage of shoots were formed by cuttings in the light experiment.

In experiment 1, the percentage of cuttings that formed new shoots tended to decrease with increasing stump heights of the stockplants from where they were taken. This is in agreement with the percentage of rooted and callused cuttings, which decreased with increasing stump heights of the stockplants from where they were taken, and with the percentage of dead and defoliated cuttings, which increased with increasing stump heights of stockplants from where they were taken. This demonstrates that new shoot formation is positively related to rooting and callusing, but negatively related to cutting mortality and cutting leaf abscission.

The low shoot formation by cuttings with increasing stump heights of the stockplants from where the cuttings were taken may be attributed to the fact that their mineral nutrient, water and carbohydrate content was too low (mainly due to competition among other shoots) for budbreak and shoot development.

In the experiment on reduced light on stockplants, the cuttings from unshielded stockplants produced significantly higher (*P* <0.05; t-test) new shoots than those from shielded stockplants did from week two throughout the four weeks of the experiment. This shows that shielding of the stockplants had a considerable effect on this trait.

The low shoot formation in cuttings taken from the screened stockplants may be attributed to the low mineral nutrient and carbohydrate contents of the cuttings during severance due to the low level of irradiance. Consequently, the current assimilate in the cuttings was not enough for rooting and new shoot development.

The results of experiment 3 showed that the cuttings from the fertilized stockplants produced a significantly higher (*P* <0.05; t-test) percentage of new shoots from week three onwards to the fourth week of the experiment. This is similar to rooting and callusing, where the higher percentage of rooted and callused cuttings were associated with the fertilized stockplants. However, percentage shoot formation is not consistent with cutting mortality and leaf abscission.

The high shoot formation results associated with cuttings from the fertilized stockplants could be ascribed to the high content of cutting nutrients during severance, such that there was enough substrates in cuttings to enhance current assimilate production for rooting and shoot production.

Number of roots per cutting

The mean number of roots per rooted cutting in experiments 1, 2 and 3 at the end of the experiments in week four ranged from 3.9 to 7.8, 2.8 to 6.2 and 4.5 to 11.5

respectively, with the highest value recorded in the fertilizer experiment. This indicates that fertilizer application to stockplants may enhance the production of more roots per cutting than the other two stockplant management factors tested here.

In experiment 1, the highest mean number (7.8) of roots per rooted cutting at the last assessment was associated with the 0.3 m height treatment, while the lowest (3.9) was recorded in the 2.1 m treatment. However, the mean number of roots per rooted cutting increased with decreasing stump heights. This observation concurs that of rooting, callusing and shoot formation, which increased with decreasing stump heights, but differs from cutting mortality and leaf abscission, which increased with increasing stump heights.

The increase in the mean number of roots per rooted cutting with decreasing stump height could be attributed to the fact that there are fewer shoots at lower stump heights competing for mineral nutrient, water and carbohydrates in the stockplant, mineral nutrients and water from the soil are more easily made available at lower heights and it is also believed that shoots from the bottom of mature trees are juvenile. Consequently, cuttings from low heights may contain more mineral nutrients, water, carbohydrates and may have a higher degree of juvenility, which may enhance the production of more roots per rooted cutting.

In experiment 2, the mean number of roots per rooted cutting was 2.8 and 6.2 in cuttings from screened and unscreened stockplants respectively. This is in accordance with observations on rooting, callusing and shoot formation, where the higher values were associated with cuttings taken from unshielded stockplants.

In the experiment on fertilizer application, a higher mean number of roots per rooted cutting was associated with cuttings harvested from fertilized stockplants. This is similar to the higher percentage of rooted, callused and sprouted cuttings, which were also associated with cuttings harvested from fertilized stockplants. However, this observation is consistent with that on cutting mortality and leaf shedding, where higher percentage values were associated with unfertilized stockplants. At the end of the experiment in week four, a mean number of roots per rooted cutting of 11.5 and 4.5 was recorded in cuttings taken from fertilized and unfertilized stockplants respectively.

CHAPTER 7

Effect of pollarding mature trees on rooting of leafy stem cuttings of *Ricinodendron heudelotii.*

CHAPTER 7

7.0 Effect of pollarding mature trees on rooting of leafy stem cuttings of *Ricinodendron heudelotii.*

7.1.0 Experiment 1: Effect of different pollard heights on rooting of leafy stem cuttings of *Ricinodendron heudelotii.*

7.1.1 Introduction

Most studies in vegetative propagation have reported difficulties associated with rooting cuttings from mature trees (Bonga, 1981; 1982; Brix, 1973; Biondi and Thorpe, 1981). As a plant grows from seedling to reproductive maturity, it undergoes morphological as well as physiological changes (Fortanier and Jonkers, 1976; Borchert, 1976; Hackett *et al.*, 1992). All these changes affect the rooting ability of cuttings taken from them. The shoots from where the cuttings are taken form secondary thickening and become so lignified that barriers which hinder root initiation and development are formed (Brix, 1973; Bonga, 1981). The leaf may some times become chlorotic and the stomatal mechanism breaks down resulting to reduced photosynthetic production of assimilates (Fortanier and Jonkers, 1976). This impairs carbon dioxide uptake and photosynthesis in these leaves resulting in rooting inhibition. At this stage of development, there is a reduction in the soluble carbohydrate, nutrient and growth regulator content of the mature shoot, all of which may adversely affect rooting of cuttings taken from them (Leakey *et al.*, 1993).

In the present study, attempts were made to root cuttings from shoots obtained from reproductively mature trees. The shoots were induced by pollarding the mature trees to different heights. The main aims of this investigation were (i) to study the influence of pollard height on rooting ability and (ii) to study the effect of pollard height on the growth and fruiting of trees raised from these cuttings. The research hypothesis that the experiment was designed to test is whether or not cuttings taken from sprout shoots at heights up to ten metres would root, and the plants raised from such cuttings would produce flowers and fruits at an earlier age. To this end, mature trees were pollarded at four different heights.

7.1.2 Materials and methods

Using local fruit collectors as guides, twenty-five trees of *Ricinodendron heudelotii* were located in the Southern Bakundu forest reserve in mid-February 1991. Some of these were stumped for experiments described earlier. In the latter part of January 1993 thirty-four more trees were sought. All the trees were numbered and their sex recorded, if known. Twenty-four of the trees were selected for pollarding at four different heights of 1.5, 3, 5 and 10 m. Six trees were pollarded to the same height in each treatment. Wooden ladders and platforms were erected around each tree at the corresponding height for ease of management of the sprout shoots at that height above the ground. In erecting the scaffolds, care was taken not to wound the stumps. The platforms were built one metre below the cut surface of each stump so as to enable shoots to sprout and grow freely (Plate 7.1 A, B, C, D & E).

The stumps all sprouted copiously. The felled trunks also sprouted in some cases. Sprout shoot production was heavy because pollarding was carried out at the start of the rainy season.

To cope with the quantity of cuttings, three additional propagators were built between the 6th and 18th of April 1993. On the 21st, four shoots were obtained from four plants of each height treatment. In addition, eight additional shoots were collected, from four of the felled trunks and the other four from the branches of four felled trunks. This gave a total of seventy-two shoots which were grouped in four

Plate 7.1: Pollarded trees of 10 m (A), 5 m (B), 3 m (C), 1.5m (D) and felled trunk (E), from which cuttings were obtained.

B


C

D


E

sets (corresponding to the four shoots per stockplant) of eighteen shoots each. Five cuttings were taken from each shoot, according to node position, from each of the eighteen sources. The leaf of each cutting was trimmed and the cutting treated with IBA as described in earlier experiments. The cuttings so treated were inserted according to node position, in one of the eighteen lines that constituted each of four blocks. Five cuttings from each of the remaining seventeen shoots were all treated in the same manner and set in each of the eighteen lines in each block. The other three sets of eighteen shoots were each treated in the same way to fill the four blocks (4 shoots per stump x 4 stumps per ht. x 4 hts. + 4 shoots from trunks + 4 shoots from branches). Two weeks after insertion, the cuttings were assessed as discussed earlier.

Light measurements were recorded as a series of spot readings by placing a quantum sensor (Skye Instrument Ltd., Llandrindod Wells, UK) in the centre of the foliage of the sprouts of each pollard at the cut surface during the period of shoot growth on the pollards. The days and time of taking light readings were not fixed since this depended on the availability of the only Research Station all-season vehicle (Toyota pick-up). The irradiance data are presented in Table 7.1 in μmol m^{-2} s^{-1}.

Table 7.1: Irradiance data of pollarded trees from which cuttings were taken for the pollard height experiment in S. Bakundu Forest Reserve, Kumba, Cameroon in μmol m^{-2} s^{-1}.

Tree number	Tree Height	Maximum irradiance	Minimum irradiance	Mean irradiance	Mean of tree height
18	1.5	388	212	244 ± 22	554 ± 26
5	1.5	725	618	664 ± 23	
15	1.5	845	538	712 ± 33	
4	1.5	730	531	593 ± 26	
1	3	301	205	257 ± 34	223 ± 27
8	3	285	215	240 ± 22	
7	3	241	155	204 ± 31	
17	3	226	160	192 ± 23	
12	5	365	246	290 ± 24	340 ± 19
9	5	236	166	195 ± 23	
14	5	220	124	166 ± 9	
16	5	748	685	708 ± 21	
11	10	175	112	150 ± 20	475 ± 28
2	10	643	563	605 ± 25	
3	10	800	523	639 ± 32	
13	10	645	291	505 ± 32	

Assessment

Assessment was made on a weekly basis for callus formation, leaf shedding, shoot formation, cutting mortality, changes in bottom and top cutting diameters and callus diameter. Daily observations were made during all the experiments for incidence of dry leaf surfaces, cutting death, leaf shedding and propagator leakages.

Analysis

The analysis of deviance by stepwise regression revealed that the rooting ability of *Ricinodendron heudelotii* was significantly affected by block, diameter at cutting base and cutting length, but was not significantly affected by pollard heights (Table A26).

Callus formation

In the first week, the percentage of cuttings that callused was 3% in the cuttings that were taken from the felled trunks. None of the cuttings in the other treatments had produced callus by this time. In the second week, the percentage of callused (20%) cuttings was significantly (P <0.05; t-test) lower in the cuttings harvested from tree branches than in the other treatments which did not differ significantly (P >0.05; t-test) from each other. In the third and fourth weeks, these differences were maintained such that the lowest proportion of callusing was recorded in cuttings from branches. There were no significant differences between the height treatments (1.5, 3, 5 and 10 m) and the trunk treatment in this variable at any time after week one (Figure 7.1b).

Cutting mortality

In the first week, 20% and 3% of cuttings in the felled branches and trunks treatment respectively died. The percentage of cutting mortality increased in all treatments between weeks one and three. In the second week, cuttings from branches and felled trunks displayed higher percentage mortalities than the other treatments, and there were significant differences (P <0.05; t-test) between these two treatments with mortality in the branch treatment being relatively high. At week four, mortality in the branch and felled trunk treatments was significantly higher than in the four height treatments (1.5, 3, 5 and 10 m), but they were not significantly (P >0.05; t-test) different from each other. Mortality rate was relatively low in the four height treatments throughout (Figure 7.1c).

Plate 7.2: Rooted cuttings from mature pollarded trees of *Ricinodendron heudelotii.*

Leaf shedding

The rate at which cuttings shed their leaves was noticeably high in this experiment, commencing in the first week, with the highest proportion of leaf shedding associated with cuttings from branches and trunks. Leaf shedding in these two treatments was significantly higher than in the other treatments in week two (*P* <0.05; t-test), and leaf shedding in the branch treatment was significantly higher than in the trunk treatment. In the third and fourth weeks, the branch treatment was associated with a higher proportion of leaf abscission than the other five treatments, which were not significantly different from one another (Figure 7.1d).

Shoot formation

No shoot formation was registered in the first week in any of the six treatments. However, some cuttings had formed new shoots in all the treatments by week two, and the percentage of cuttings forming new shoots increased rapidly between the second and third weeks. At the end of week four, percentage shoot formation was significantly higher (*P* <0.05; t-test) in the 1.5 m and 10 m treatments than in the others. Shoot formation in the branch treatment was significantly lower than the other treatments after four weeks.

Number of roots per rooted cutting

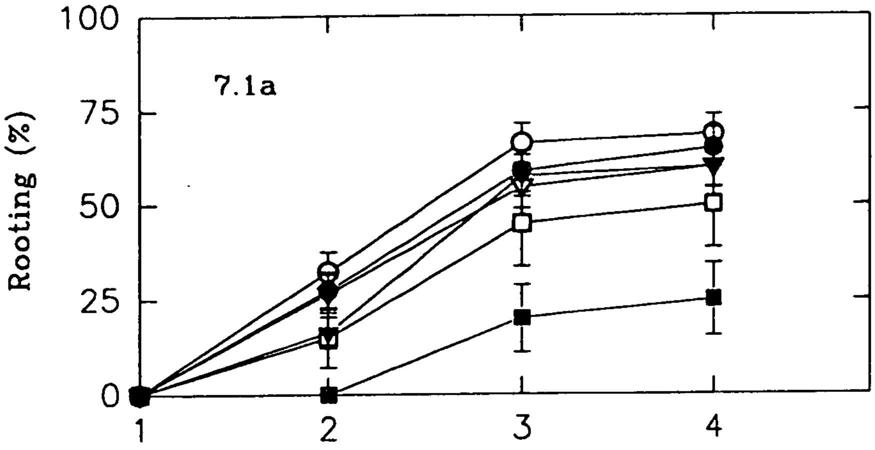
After four weeks, mean root number per rooted cutting in cuttings from the 1.5 m treatment was significantly (*P* <0.05; t-test) higher than in those from the other pollard heights, branches and trunks. The mean root number per rooted cutting in the 1.5 m treatment was 6.5. The branch treatment was significantly lower than the other treatments, but there were no significant differences (*P* >0.05; t-test) between the 10 m, 5 m, 3 m and trunk treatments. Generally, the mean number of roots per rooted cutting tended to increase with decreasing pollard height, although the trend was not pronounced (Figure 7.1f).

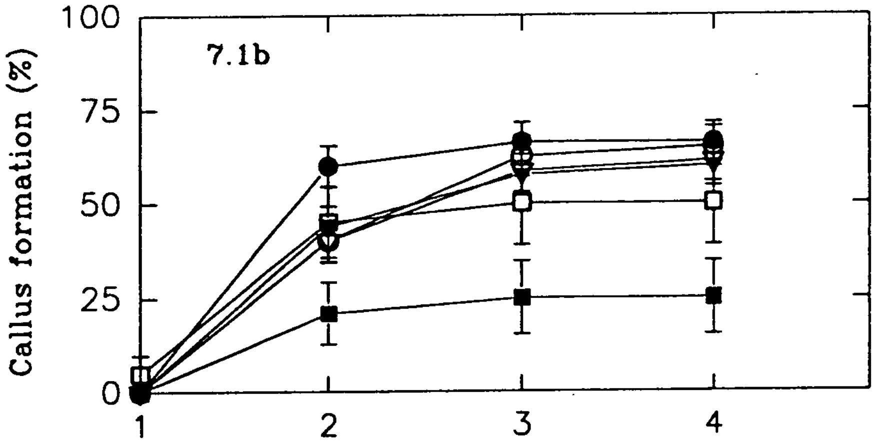
7.2 Discussion

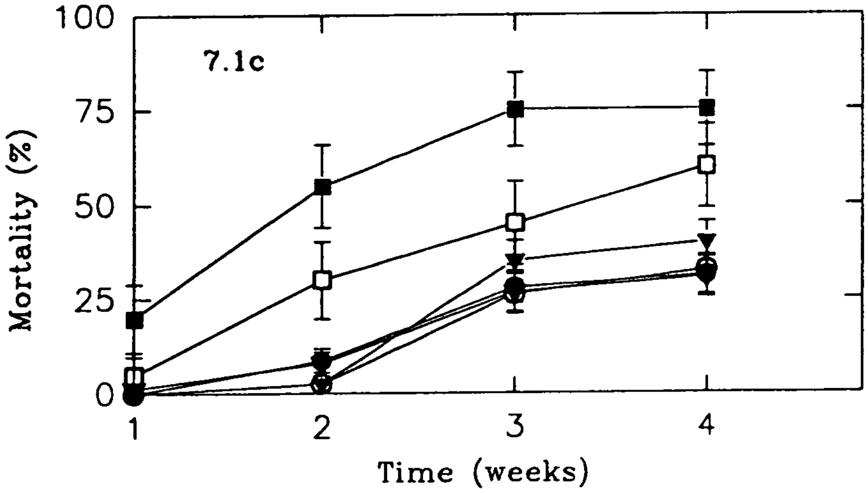
Percentage rooting

It has been demonstrated previously that the rooting ability of cuttings declines with increasing maturity of the stockplants from where they are taken (Fortanier and Jonkers, 1976; Bonga, 1982; Hackett, 1985). Stockplant maturity increases with height, such that the crown is believed to be the mature zone while the base is the juvenile zone of the same plant (Borchert, 1976; Hackett, 1988; Bonga, 1982; Komissarov, 1969). In this regard, decline in the rooting ability of cuttings with increasing tree age has been attributed to the loss of juvenility in shoots originating from the upper portions of the stockplant (Leakey *et al.*, 1992). However, there are young shoots in the mature crown of a large tree just as there are old tissues in its juvenile base or stump (Leakey *et al.*, 1992). Shoots of the same chronological age, irrespective of their location on the stockplant, may produce easily rooted cuttings if they are physiologically in the appropriate condition (Fortanier and Jonkers, 1976). This hypothesis agrees with the observation of Borchert (1976) that flowering shoots may root easily, or shoots with juvenile foliage may root poorly or not at all. Conversely, precocious flowering on shoots bearing juvenile foliage has been observed many times (Doorenbos, 1965, cited by Borchert, 1976).

This suggests that any decline in the rooting ability of cuttings that is attributed to chronological ageing can be reversed, while that related to ontogenetic ageing (ageing from seed germination to sexual maturity and death) may be difficult, since the former is more linked with the physiological state and factors such as nutrient, water, auxin status, lignification and microclimate of the plant organs (shoots), which can easily be manipulated by the propagator (Leakey *et al.*, 1992).


7.1a
Rooting (%)
100
75
50
25
0
1
2
3
4


7.1b
Callus formation (%)
100
75
50
25
0
1
2
3
4


7.1c
Mortality (%)
100
75
50
25
0
1
2
3
4
Time (weeks)

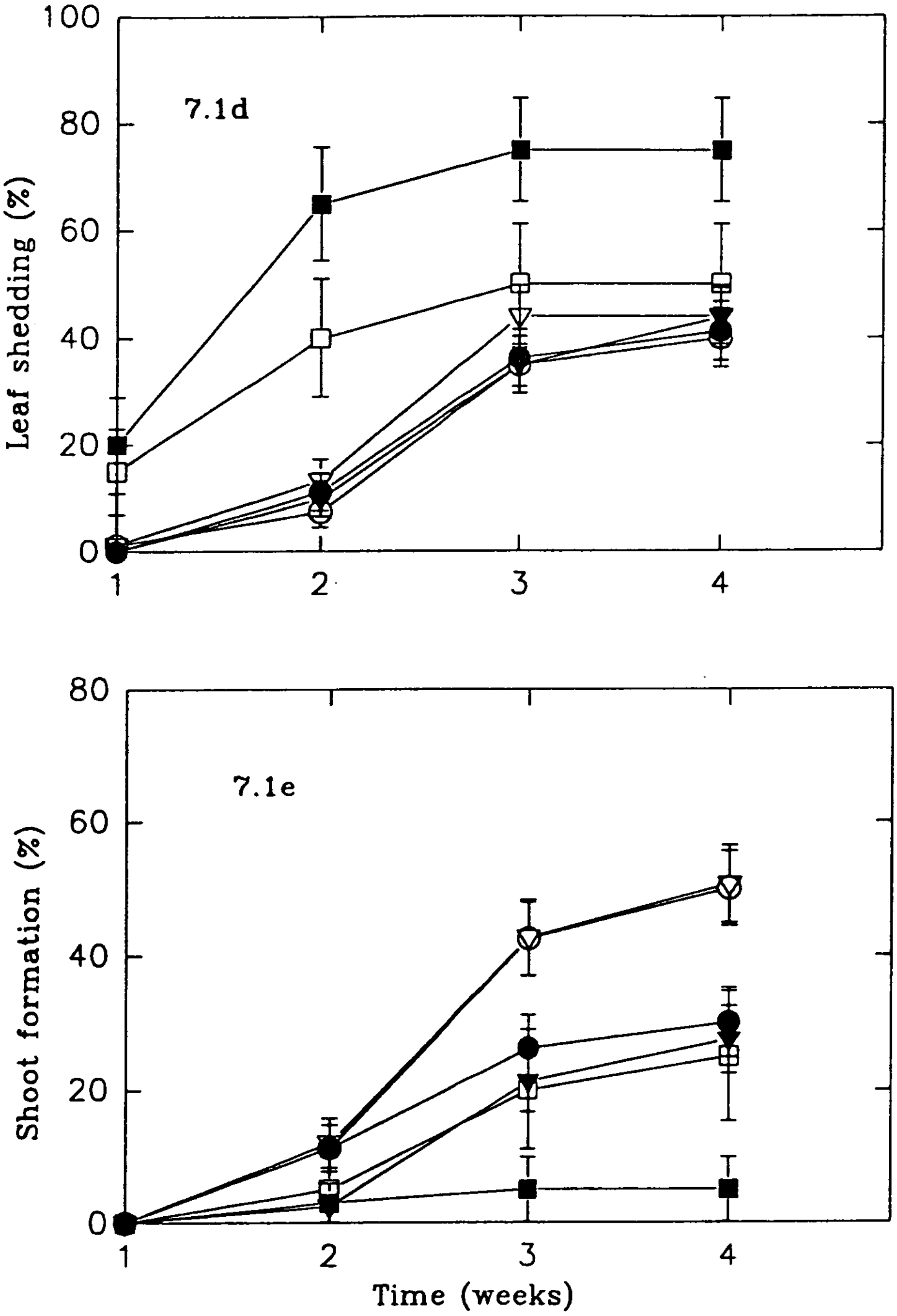


Figure 7.1: Effect of pollard heights on rooting of leafy stem cuttings of *R. heudelotii*, (○ = 1.5 m ● = 3 m, ▼ = 5 m, ▽ = 10 m, □ = trunk and ■ = branch). Bar = ± SE.

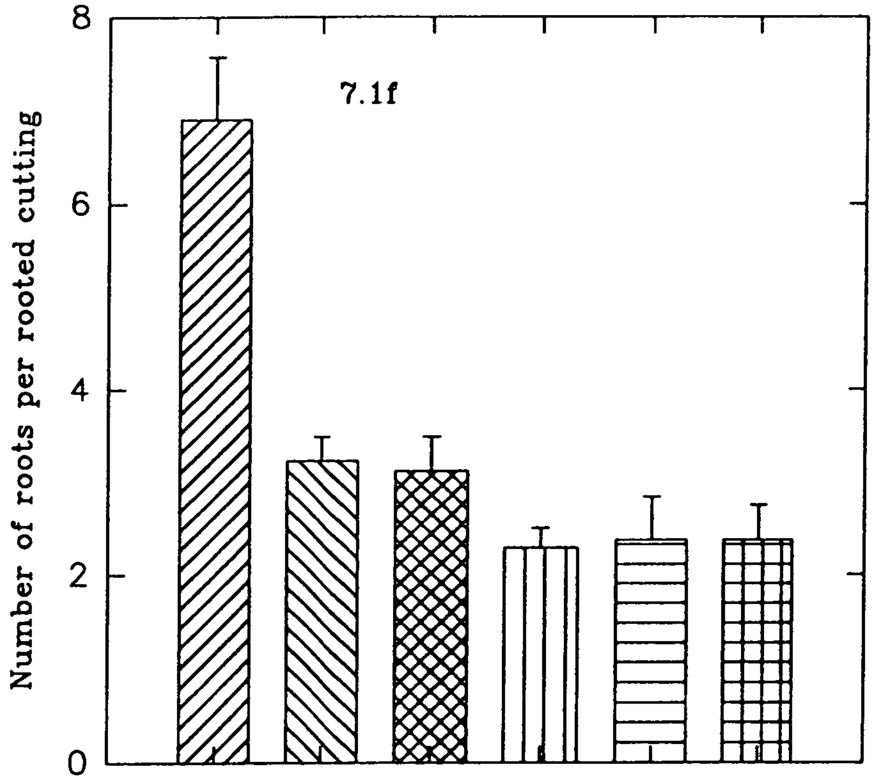


Figure 7.1f: Effect of pollard heights on number of roots per rooted cutting of *R. heudelotii*. (▨ = 1.5 m, ▧ = 3 m, ▩ = 5 m, ▥ = 10 m, ▤ = branch and ▦ = trunk) (after 5 weeks). Bar = + SE. (n = 80).

In the present study, the percentage of rooted cuttings in all the four height treatments at the end of the experiment in week four ranged from 60.0% to 68.8% in the 1.5 m and 10 m height treatments respectively. No significant differences were observed between the height treatments at end of the experiment. This is in agreement with the hypothesis that cuttings of the same chronological age, irrespective of their location on the stockplants, may root differently only when and if they are physiologically different (Fortanier and Jonkers, 1976). In this experiment, the shoots from where the cuttings were taken were of the same chronological age and it is assumed that the cuttings were physiologically in a similar condition (see materials and methods).

The percentage of rooted cuttings decreased with increasing pollard heights, with the highest value of 68.8% associated with the 1.5 m height treatment, while 60.0% was recorded in the 10 m height treatment, although this was not significant. These results are different from those of other species such as ***Triplochiton scleroxylon*** (Nketiah, 1994), where the percentage of rooted cuttings was inversely related to four stockplant heights of 0.5 m, 1.0 m, 1.5 m and 2 m. In cone spruce (Grace, 1939, cited by Komissarov, 1969), percentage rooting was 75% for cuttings from lower as against 43% for those from the upper crown height. This also concurs with the observation of Morgan and Mason (1992) that the rooting ability of cuttings harvested from Sitka spruce hedge declined with increasing heights to which the hedge was pruned. This phenomenon was observed on seedling stockplants of *Triplochiton scleroxylon* cut to heights up 1.5 m, where cuttings from basal shaded shoots rooted considerably better than more apical shoots (Leakey, 1983).

The increasing tendency displayed by the percentage of rooted cuttings with decreasing stockplant heights as exhibited by the cuttings in this experiment may be ascribed to juvenile shoots arising from adventitious buds laid down early in the life cycle of the plants that have remained suppressed (arrested juvenility) at the base of the stockplant up to the time of stumping (Bonga, 1982; Hackett, 1988; Fortanier and Jonkers, 1976). Consequently the tissues of the cuttings from this point have juvenile characteristics such as the ability in somatic self duplication, high content of auxin, auxin co-factors, enzymes, enzyme-co-factors, water, mineral nutrients, appropriate phyllotaxis, leaf shape, leaf retention and genetic stability (Hackett, 1988; Bonga, 1982), which all enhance rooting. The high rooting ability of cuttings from the base of the pollards may be attributed to shoots arising from buds close to the root-shoot junction, which may contain arrested juvenile buds, easy to flush into juvenile sprouts if the tree is stumped or severely pruned (Bonga, 1982; Brix, 1973; Wiltshire and Reid, 1992). The length of the internal transport system of the stockplant is nearer the shoots at the base than those further away from the source of water and mineral nutrients (soil and roots) (Fortanier and Jonkers, 1976), and this might have enhanced the percentage of rooted cuttings at the lowest 1.5 m height treatment and disfavoured the others from the 3 m to the 10 m height treatments. The number of shoots which increased with increasing stump heights (Figure 7.1g) might have caused more competition among the shoots at the higher stump height treatments than at the lower height treatment of 1.5 m. This might have in turn caused differential nutrient exhaustion in cuttings from the heights with relative shoot numbers to exhibit the rooting pattern recorded here. This is consistent with the observation of Leakey (1983) that the negative relationship between the rooting ability of the cuttings of *Triplochiton scleroxylon* and the stockplant heights from where they were harvested appeared to be related to competition between the shoots.

Variation in the microclimatic conditions of the stockplants imposed by the overhead crowns of the nearby trees in the reserve might have provided adequate shading that favoured the rooting of cuttings from the lower stumps than in taller ones. Cutting diameter and cutting length were shown by analysis of deviance for stepwise regression to significantly affect the rooting ability of cuttings in this experiment. This demonstrates that cutting diameter and shoot internode varied considerably at different pollard heights. Changes in cutting lengths with stump height of stockplants from where they are taken may cause a variation in their carbohydrate, starch, minerals and auxin content, which can affect their rooting ability. Variation in leaf area on shoots at different stockplant heights may also be implicated in the variation in the percentage of rooted cuttings taken from them (Borchert, 1976). Leaves which were attacked by perforating insects and or other pathogens and were left with incomplete and ineffective leaf areas might have also caused a variation in the percentage of rooted cuttings at the corresponding stump height.

Callus formation

Except for cuttings from the felled trunks where 5% had callused by the end of the first week, the rate of callus formation was the same in the other treatments, all commencing in the second week after insertion. At the final assessment at week four, 5.8% of the cuttings did not callus but rooted in the lowest height treatment of 1.5 m, while all the cuttings that callused in the highest height treatment of 10 m rooted. This suggests that the rooting of some of the cuttings in the lowest height treatment was not dependent on callus formation, in conformity with the view of Hartmann and Kester (1983) and Van de Lek (1925, cited by Komissarov, 1969), that root formation in cuttings during propagation is not connected with the activities of callus production. The fact that all the callused cuttings in the tallest height treatment of 10 m rooted demonstrates that rooting in the cuttings from this height are highly dependent on callus formation. This is contrary to the observation made by Nketiah (1994) that most of the cuttings of *Triplochiton scleroxylon* did not produce callus, but rooted.

In the 3 m and 5 m treatments, 2.0% and 2.2% of the cuttings that callused in each case, did not root. This might have been due to excessive callus formed, most probably, by all the living tissues of the bark, wood and pith of the cuttings, which might have hindered root formation in these cuttings (Komissarov, 1969).

Cutting mortality

In this experiment, the time of cutting mortality varied considerably among the six treatments, such that by the first week after cutting insertion, 9%, 5% and 1.3% of the cuttings in the branch, trunk and 5 m height treatments had died, while cutting mortality started in the other treatments in week two of experiments. There were no significant differences in the percentage of cutting mortality among the four pollard height treatments. Values of percentage cutting mortality in these treatments were 40.0%, 30.7%, 31.3% and 32.5% associated with 10 m, 5 m, 3 m and 1.5 m respectively. These results conform with those obtained from different stump heights of *Triplochiton scleroxylon* by Nketiah (1994), who recorded high cutting mortality (69.1% the 2 m stump height and a low value (41.7%) in the 0.5 m stump height. Cutting mortality was negatively related to rooting and callusing in this experiment.

The cutting mortality associated with the highest pollard height of 10 metres may be attributed to the fact that there were many shoots (mean of 18.5) (Figure 7.1g) competing for the available nutrients, similar to the competition observed among many shoots on the stump of *Triplochiton scleroxylon* (Leakey, 1983). This could lead to low nutrient content in the cuttings that might have decreased their survival rate in the rooting medium. At the height of 10 metres, by necessity, positional differentiation between shoots, and variation in the angle of growth of each shoot may occur due to the increased number and complexity of shoots. This increase in morphological complexity must be accompanied by increasing complexity of distribution and transport of substances such as current assimilate, auxin, mineral nutrients and water required for growth and subsequent rooting. In addition, problems of supra and suboptimal availability of carbon dioxide, leaf size and shape, net photosynthesis, stomatal distribution and density, internode length, shoot position, the degree of lignification and the node position of the cuttings during severance could contribute to cutting mortality during rooting (Leakey *et al.*, 1992; Phillips, 1975; Borchert, 1976). The great distance away from the roots and the base of the stockplant as a whole could adversely affect the internal transport system by limiting the supply of water and mineral nutrients to the cuttings harvested from that height (Fortanier and Jonkers, 1976). Such cuttings may not survive the rooting period unless net photosynthate is enough to keep them alive and to enable them to root. The mineral nutrient reserves, auxin, carbohydrate, enzyme and rooting co-factor content of the branches and trunks may be so depleted by the new shoots that cutting from those sources may root poorly or not always root.

Leaf abscission

At the end of the experiment in week four, the highest proportion of cuttings that shed their leaves was associated with the branch treatment, which was significantly higher ($P < 0.05$; t-test) than other treatments in this variable. There were no significant differences between the other five treatments. Leaf shedding and cutting mortality were both negatively related to rooting and callusing, but positively related to each other. Among the different pollard height treatments, the percentage of cuttings that shed their leaves increased with increasing pollard heights. This is in accordance with the observation made by Nketiah (1994) that the percentage of leaf abscission of cuttings from different stump heights increased with decreasing heights of *Triplochiton scleroxylon* stockplants. The decreasing tendency in leaf abscission with decreasing pollard height is in conformity with two hypotheses: (1) that the cuttings at the base of the stockplant are juvenile and (2) that juvenile cuttings tend to retain their leaves during propagation (Bonga, 1982; Borchert, 1976; Hackett, 1992).

The fact that leaves senesce in mature shoots and tend to be retained in juvenile ones is not surprising because leaf abscission is related to plant maturity, which is in turn characterized by flowering and fruiting. At fruiting time, many organs in the plant, particularly in the crown for most plants, are competing for nutrients, such that nutrients are withdrawn or diverted from older ones to the flowers, fruit and other meristematic tissues (Salisbury and Ross, 1978). This is generally the onset of senescence. When nutrients are withdrawn from the leaf because the shoot is ageing, and has to bear fruits, dry up and drop in the process of self pruning, then messages, mediated through hormones such as abscisic acid and ethylene are transmitted to stimulate the withdrawal, breakdown or synthesis inhibition of chlorophyll, RNA, proteins and nutrients from the leaves followed by abscission (Leopold and

Kriedemann, 1975). Prior to leaf senescence and abscission, an abscission layer is formed across the petiole near its junction with the stem under enzymatic activities (Kozlowski,1973), to protect the cuttings or shoot from fungal or bacterial infection.

Although of the same chronological age, cuttings within the same shoot and between different shoots may vary considerably (Leakey *et al.*, 1992). The starch and sugar content of cuttings may decrease with the distance of the shoot away from the base, resulting in leaf shedding. Similarly, the content of auxins and other growth regulators may decrease in shoots produced further away from the stem base, thereby reducing the efficiency of the cuttings and resulting to leaf shedding. Mutual shading of shoots and shading by nearby forest trees in the reserve might have reduced the photosynthetic efficiency of the leaves of some of these cuttings resulting in leaf shedding during propagation.

Shoot formation

In contrast to leaf abscission, shoot formation started in this experiment in week two, with the lowest percentage of shoots being formed in the branch treatment. By the end of the experiment in week four, the proportion of cuttings that formed new shoots displayed a considerable variation with pollard heights, with the highest value of 50.0% and 50.7% recorded in the 1.5 m and 10 m height treatments respectively and the lowest value of 5.0% associated with the branch treatment. The highest percentage of rooted cuttings was also recorded in this treatment, suggesting that the cuttings from the 1.5 m height had sufficient carbohydrates, auxin, water and other mineral nutrients to enable the highest percentage shoot and root formation. This may be ascribed to the presence of leaves on the cuttings, since the lowest proportion of cuttings from this height shed their leaves, and leaves are known to produce current assimilates, endogenous auxins, enzymes and co-enzymes during propagation (Newton *et al.*, 1992b; Leakey and Coutts, 1989, Haissig, 1986).

Number of roots per cutting

At the end of the experiment in week four, the mean number of roots per rooted cutting was highest (7.1) in the cuttings from the lowest pollard height of 1.5 metres. This was followed by the 3 m and 5 m pollard height treatments, with values of 3.1 and 3.3 respectively. The lowest mean number of roots per rooted cutting was recorded in the highest pollard (10 m) height. Except for the cuttings from the branch and trunk treatments, the mean number of roots per rooted cutting tended to increase with decreasing heights in the four pollard height treatments. This observation is consistent with rooting, callusing and shoot formation where the highest value was associated with cuttings from the lowest pollard height treatment.

The leaves retained by cuttings which are juvenile by virtue of the 1.5 m height (base) of the stockplants from where they are taken (Borchert, 1976), produce and supply carbohydrate to the cutting tissues at the rooting zone (Ahmad *et al.*, 1990). Leaves also produce endogenous auxins, enhance the uptake of supplied auxins (Jarvis, 1986) and co-factors and synergists (Staden and Harty, 1988), which are loaded, and transported for efficient delivery for more and increased root formation at the cutting base (Jarvis, 1986). All these activities might have resulted in the high mean number of roots per rooted cutting associated with the cuttings from the 1.5 m pollard height of this experiment.

CHAPTER 8

General Conclusion and Future Research

CHAPTER 8

GENERAL CONCLUSIONS AND FUTURE RESEARCH

Introduction

The present study set out to address two main issues: using the low-technology non-mist propagation system, (1) can the multipurpose trees species sought after by the rural farmers in Cameroon natural forests be selected and domesticated by rooting leafy stem cuttings, and (2) can the domesticates capture and sustain the superior genotypes from wild populations of these non-timber species and be made available to the farmer for introduction into farming systems for increased and improved yields. This chapter summarizes the overall conclusions of the experiments described previously.

Irvingia gabonensis, Ricinodendron heudelotii and *Gnetum africanum* are three of the many multipurpose plant species in the natural forests of Cameroon that were propagated vegetatively by rooting leafy stem cuttings in this study. It is hoped that many others (there are about 151 multipurpose plant species in the Nigerian and Cameroonian forests, Okafor, 1978) will be propagated during the big project in the future, using this technique. Successful rooting of the cuttings of these species depended on a complex interplay of genetic, physiological, morphological and environmental factors before and after cutting severance from the stockplants.

The three species did not display marked variations in their response to similar treatments, although some contrasting responses were observed. This suggests that the ecological source of plant material within this zone will not pose any problems of ecological adaptation for future propagation work.

In an effort to understand the physiological requirements for the rooting of the cuttings of these species, some factors, both pre- and post-severance, which have been known to have crucial effects on the rooting ability of leafy stem cuttings were studied. They are discussed below.

Pre-severance stockplant management

Shading

Stumped stockplants were shaded with light filters in order to modify their physiology and morphology, and to study the influence of this modification on the rooting ability of cuttings taken from them. In a similar context, Tchoundjeu (1989) planted *Leucaena leucocephala* as shade in rows on either side of hedged *T. scleroxylon* stockplants, and achieved an increased percentage rooting of the cuttings taken from them from 21% to 74%. However, in the present study, the percentage of rooted cuttings from the unshaded stockplants was significantly higher ($P < 0.05$) than that of cuttings from the shaded stockplants, although ANOVA test showed no significant differences between the two treatments (Table A21) due to unassigned variation. This contradicts the hypothesis that the percentage of rooted cuttings is higher if they are harvested from stockplants grown at lower irradiance than at higher irradiance (Hartmann and Kester, 1983; Hansen and Ericksen, 1974; Hansen, 1976). However, the optimum irradiance for propagation varies from species to species. Increased irradiance may inhibit or delay rooting, promote rooting or have no effect on cuttings (Moe and Andersen, 1988). The percentage of rooted cuttings from unshaded stockplants was 86% as against 61% from shaded stockplants in the present study. It is possible that from a range of three to five shading treatments, an optimum shading requirement to produce cuttings that can give higher than 86% rooting may be defined for this species (Moe and Andersen, 1988).

Fertilizer (NPK) application

The cuttings from the stockplant stumps on which the NPK fertilizer was applied registered a significantly higher ($P < 0.05$; Table A24) rooting percentage than those from the control stockplants. Similarly, the mean number of roots per rooted cutting from the fertilized stockplants was significantly higher than that from the unfertilized stockplants. This is in agreement with Moe and Andersen (1988) who reported that fertilized outdoor ornamental species produced a higher percentage of rooted cuttings than those from stockplants which were not fertilized. However, a high NPK fertilizer application to the stockplants of *A. guachapele* (Mesén, 1994) had a negative effect on their growth and reduced the subsequent percentage of rooted cuttings taken from them, and it seem likely that NPK application rates may sometimes have been supraoptimal (Moe and Andersen 1988). The growth of the fertilized stockplants of *R. heudelotii* and the subsequent rooting ability of the cuttings harvested from them was not adversely affected by the fertilizer treatments in this experiment. This is an indication that the mineral nutrient in the soil where the stockplants were planted was low. It is possible that an even higher rate of NPK application could increase subsequent rooting percentage, root quality and number of roots per rooted cutting. Ideally, an experiment should be carried out with a range of nutrient supply to determine optimal conditions.

The significantly lower rooting percentage (67%) associated with cuttings from the unfertilized stockplants could be attributed to the low mineral nutrient availability in the soil where the stockplants were planted. The nutrient contents of the cuttings during severance could have consequently been low. Failure of cuttings with low nutrient content has previously been attributed to low auxin, photosynthetic efficiency or hydrolytic enzymes for rooting, and to an inefficient translocation of materials to the rooting zone at the cutting base (Haissig 1986; Moe and Andersen, 1988; Davis; 1988). Further work should include measurement of foliar nutrient content of the stockplants to detect any deficiency.

Stump height

The physiology, morphology and subsequent rooting potential of a cutting is influenced by the position within the shoot from which it is taken (Leakey and Coutts, 1989) and the position of the shoot within the stockplant canopy (Leakey, 1983; Leakey, 1985; Tchoundjeu, 1989). The percentages of rooted cuttings from the three height treatments (0.3 m, 1.2 m and 2.1 m) in this experiment were found to be significantly different ($P < 0.01$) from each other. The percentage of rooted cuttings was negatively correlated with the stockplant height from which they were taken. Similarly, the mean number of roots per rooted cutting displayed the same pattern of behaviour. These results conform with those of Nketiah (1994), who found very similar response in *T. scleroxylon* using four different stump heights (0.5 m, 1.0 m, 1.5 m and 2 m).

Sapling stockplants of *R. heudelotii* were cut down to form stumps with heights ranging from 0.3 m to 2.1 m. The aim was to attempt to root mature plant tissue, with the idea that this material would retain its mature state. This follows the concept that tree species are more mature towards the crown than they are at the base (Hackett, 1992; Borchert, 1976; Fortanier and Jonkers, 1976; Bonga, 1982). Whether or not the cuttings that were rooted from the more mature trees did retain a degree of reproductive maturity remains to be tested.

Pollard height

There were significant differences ($P < 0.001$) in rooting of cuttings from the different pollarding treatments. The lowest percentage rooting was associated with the cuttings taken from branches. This is consistent with experimental evidence that it is more difficult to root cuttings from tree crown branches than it is to root those from basal shoots (Borchert, 1976; Bonga, 1982; Hackett, 1994;). The percentage of rooted cuttings from the felled trunks was not significantly different ($P > 0.05$; t-test) from that of the height treatments of 3 m, 5 m and 10 m. However, the use of felled tree cannot, generally, be recommended as it is likely that cuttings from them may be suffering from a deficiency of nutrients. In the present case, the leaves appeared chlorotic, probably due to a lack of nitrogen (Blazich, 1988; Salisbury and Ross, 1978). It is possible that this could impair photosynthesis in the leaves leading to low carbohydrate, auxin and enzyme contents, a delay in establishment and a weak plant. Low nutrient contents could be attributed to the depletion of reserves in the trunk after felling.

The percentage of rooted cuttings from the four pollard height treatments (1.5 m, 3 m 5 m and 10 m) did not differ significantly from each other, with the highest percentage of rooted cuttings recorded in the lowest pollard height of 1.5 m. This is in agreement with the findings of many workers on juvenile-mature plant tissues and rooting, such as Borchert (1976), Fortanier and Jonkers (1976), Bonga (1982) and Hackett (1988), who reported that cuttings from the basal shoots of a plant provide a higher percentage of rooted cuttings than those from shoots or branches at the crown level.

This trend of rootability with height of the stockplant (for pollards) was also seen when the stockplants were in the form of coppiced saplings at heights of 0.3 m, 1.2 m and 2.1 m. Very similar results on *T. scleroxylon* have been obtained (Nketiah, 1994) with stockplants cut to four different heights (0.5 m, 1.0 m, 1.5 m and 2 m). Similarly, Leakey (1989) demonstrated that the percentage of rooted cuttings of *Triplochiton scleroxylon* declined in pruned stockplants of increasing height, and was related to the number of shoots per stockplant. This negative correlation between percentage of rooted cuttings and stockplant height was attributed to competition between shoots. In fact, the number of shoots per stumped stockplant increased considerably with increasing stump height (Figure 7.g) Further experimental evidence suggested that on grown stockplants of *T. scleroxylon*, non-vertically basal shoots rooted better than apical shoots irrespective of competition from apical shoots, while rooting ability of apical shoots was markedly affected by competition from basal shoots (Leakey, 1985).

One possible reason for poor rooting from the taller and larger plants is the influence of competition. In such larger stockplants, there are always many more shoots, and thus competition for resources is intense. Cuttings from such stockplants may be, consequently, deficient in nutrients, water and growth substances. On the other hand, the reduction in rooting from large trees could be due to the mature state of that part of the stockplant where the shoots were located (Borchert, 1976). The shoots at that height and those at the base have the same chronological age but may differ physiologically (Leakey, *et al.*, 1992; Hackett, 1992). Although of the same chronological age, upper shoots may have developed mature characters earlier than basal ones (Hackett, 1992). In addition, high irradiance at the top of the tree could enhance high rates of production of photosynthate, which might have increased foliar starch contents in the cutting, thereby suppressing its ability to photosynthesize and root while in the rooting medium (Leakey and Storeton-West, 1992). Thus, many factors may have contributed to the reduced rooting in this experiment.

Post-severance treatments

Rooting media

Rooting media have been considered as an integral part of a vegetative propagation system (Hartmann and Kester, 1983; Loach, 1988). The fundamental requirements of an ideal medium are (1) porosity for aeration (2) high water-holding capacity (3) neutral pH (6.5-7.5) (4) an optimum air:water ratio (5) freedom from harmful pathogens (O'Dell and Stoltz, 1978; Hartmann and Kester, 1983; Loach, 1985; Park and Andersen, 1989). The media tested in this experiment (river sand, rotted sawdust and mixtures thereof) were chosen on the basis of local availability and low cost, and their likelihood of fulfilling the requirements of an ideal medium.

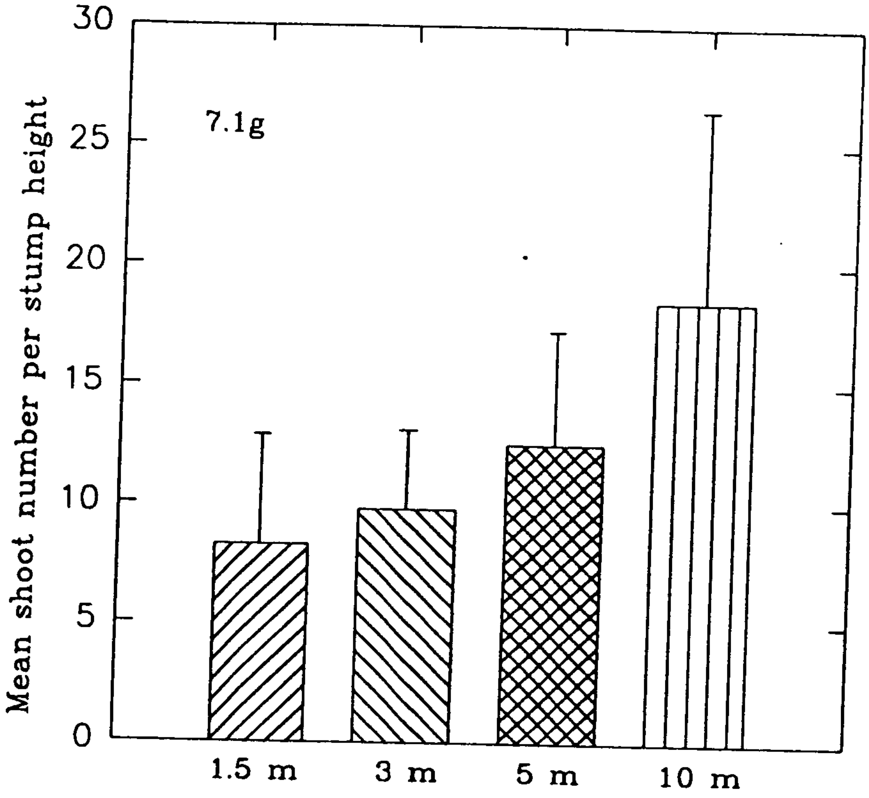


Figure 7.1g: Effect of pollard height on shoot production on the stumps of *R. heudelotii*. Bars = + SE.

The percentage of rooted cuttings of *Ricinodendron heudelotii* was significantly different (*P* <0.001) between the six rooting media treatments in this experiment. The highest percentage (82%) of rooted cuttings was associated with sawdust and this was closely followed by fine sand with 73% rooted cuttings. Similarly, the highest mean number of roots per rooted cutting was also recorded in these two media (Figures 3.1a, 3.1f). In addition, the highest proportion of cuttings that callused and formed shoots and new leaves was registered in the sawdust medium.

The response of *Irvingia gabonensis* to the six media treatments was similar to that of *R. heudelotii*. The proportion of rooted cuttings of this species was significantly different (*P* <0.001) in the six media. The highest percentage of rooted cuttings (93%) and the highest mean number of roots (7.8) per rooted cutting were recorded in sawdust. This was closely followed by fine sand and sawdust in which 79% of the cuttings rooted and in which the mean number of roots per rooted cutting was 3.2. Similarly, the highest proportion of cuttings that callused was associated with the sawdust medium.

In *Gnetum africanum*, the rooting percentage was significantly different (*P* <0.05) in the six media treatments. The highest percentage (82%) of rooted cuttings was associated with sawdust. Similarly, the highest proportion of callused cuttings was registered in the same medium. However, the highest mean number of roots (27) per rooted cutting of this species was recorded in fine sand in accordance with Mesén (1994), who registered the highest mean number of roots (7) per rooted cutting of *Cordia alliodora* in fine sand.

In terms of the proportion of rooted cuttings, *R. heudelotii*, *I. gabonensis* and *Gnetum africanum* responded similarly to the different media tested. Interestingly, variation in rooting percentage in the six media tested was quite high, ranging from 48% to 82% in *R. heudelotii*, 45% to 93% in *I. gabonensis* and 43% to 82% in *G. africanum*. This contrasts with the rooting results of *Lovoa trichilioides* in a media experiment, where the range in rooting percentage was much lower, ranging from 45% to 50% (Tchoundjeu, 1989). The lowest rooting percentage of *R. heudelotii* was associated with the mixture of gravel and sawdust, that of *I. gabonensis* with medium sand and that of *G. africanum* in the mixture of gravel and sawdust.

The fact that the highest rooting percentage and the highest mean number of roots per rooted cutting of these species was recorded in sawdust is in agreement with other tropical species, such as *Milicia excelsa* (Ofori, 1994). However, the lowest rooting percentage and the lowest mean root number per rooted cutting of *Cordia alliodora* (Mesén, 1994) were recorded in sawdust. This and the fact that the three species in this experiment rooted differently, even in sawdust, confirms the hypothesis that different species root differently in the same medium (Loach, 1985; Loach, 1988; Leakey *et al.*, 1990). There is also the possibility that the different results of different workers can be explained on the basis of differences in the type of sawdust used, as pointed out earlier, (see discussion Chapter 3). Some may contain inhibitory chemicals and not all samples may have been stored in the open, a practice that permits leaching to occur.

The high rooting percentage registered by the three species in sawdust may be attributed to its relative high air:water ratio. This enabled the medium to remain moist and thus the cuttings were not subjected to desiccation. Generally, the optimum rooting media mixture for leafy stem cuttings is a function of a combination of water holding capacity, air content, nutrient balance, water tension, gas volume, physical stability and particle size (O'Dell and Stoltz, 1978; Hartmann and Kester, 1983; Loach, 1985; Loach, 1988; Park and Andersen, 1989). Many of these factors may have contributed to the relative success of sawdust in these experiments.

Auxin application

Auxin has a central role in the initiation and development of adventitious roots in leafy stem cuttings (Hartmann and Kester, 1983; Jarvis, 1986; Haissig, 1986). Auxins have been known to hasten root initiation, promote root elongation, increase root number and quality per cutting (Hartmann and Kester, 1983; Jarvis, 1986; Blazich, 1988). Auxins are implicated in the division of the first root cell initials, synthesis of hydrolytic enzymes, mobilization of mineral nutrients and carbohydrates into the base of cuttings during root initiation, and exogenous auxins may sometimes enhance the production of endogenous auxins (Nanda *et al.*, 1968a; Rana *et al.*, 1987; Middleton, Jarvis and Booth, 1980; Blazich, 1988). Different plant species have different auxin requirements, and the optimum auxin concentrations of most plant species, particularly tropical tree species are unknown.

In the present study, the proportion of rooted cuttings of ***Ricinodendron heudelotii*** was not significantly different ($P > 0.05$) from each other among the four auxin concentrations (0 μg, 8 μg, 40 μg and 200 μg). The rooting percentage ranged from 60% in the 200 μg to 88% in the 8 μg treatments. Except for the control treatment, the rooting percentage tended to increase with decreasing auxin concentrations. Similarly, the percentage of cuttings that had produced new shoots increased with decreasing auxin concentration. These two traits contrast with the mean number of roots per rooted cutting, which exhibited an increasing trend with increasing IBA concentration.

Auxin concentration also did not have a significant effect on the rooting ability of *Irvingia gabonensis*. However, the rooting percentage ranged from 32% in the control (0 μg) to 66% in the 200 μg treatments. Except for the 250 μg treatment, with 56% of rooted cuttings, the rooting percentage increased with increasing auxin concentration. This contrasts with the case of *R. heudelotii*, where the rooting percentage was negatively related to the auxin concentration. The rooting trend was also not consistent with the mean number of roots per rooted cutting, which displayed considerable variation between the five treatments. However, the highest mean number of roots per rooted cutting was associated with the treatment in which the highest rooting percentage was recorded (200 μg).

The rooting percentage in *Gnetum africanum* was not significantly different in the five treatments. However, the rooting percentage increased somewhat with increasing IBA concentration, such that the highest rooting percentage was recorded in the 250 μg treatment, while the lowest proportion of rooted cuttings was associated with the control (0 μg) treatment. On the other hand, the mean number of roots per rooted cutting increased significantly with increasing auxin concentration. In this regard, *G. africanum* is similar to *R. heudelotii*, where roots per rooted cutting increased with increasing auxin doese, but differs from *I. gabonensis*, where no definite pattern was exhibited by increasing auxin doses and increasing number of roots per rooted cutting.

The rooting ability of the three species in this study was not affected by auxin application and even the untreated cuttings recorded a high percentage rooting as indicated earlier. This tendency to root easily has been noted in *Shorea macrophylla* (Lo, 1985), *Vochysia hondurensis* (Leakey, *et al.*, 1990), *Hopea odorata* (Hamzah, 1991), *Prosopis juliflora* (Dick *et al.*, 1991), and *Lovoa trichilioides* (Leakey, 1994, pers. comm.), all of which were successfully rooted without auxin application. In terms of the rooting percentage increasing with increasing auxin concentration, *I. gabonensis* and *Gnetum africanum* are similar to *Cordia alliodora*, *Albizia guachapele* and *Vochysia hondurensis*, where percentage rooting increased with IBA concentration up to an optimum value for each species and then declined with increasing auxin dose (Leakey, *et al.*, 1990). Contrary to the above, the rooting percentage of *Ricinodendron heudelotii* cuttings decreased with increasing auxin concentration. The fact that the rooting percentage of *G. africanum* did not decline at the highest auxin concentration of 250 μg indicates that the optimum auxin dose for maximum rooting may be above 250 μg. The results also show that the optimum auxin dose for *R. heudelotii* is between 8 μg and 40 μg and that doses above this, up to 200 μg, reduced rooting considerably. The optimum dose for *I. gabonensis* is likely to be between 150 μg and 200 μg, since the 200 μg dose gave only 66% rooting and the 250 μg dose reduced rooting. The knowledge of the optimum auxin requirements, which may just be below the toxic point, (Hartmann and Kester, 1983), is of practical importance as it may increase cutting production, save time and reduce the cost of propagation.

The reasons why the cuttings of different species have different requirements with respect to auxin concentration are poorly understood (Leakey, 1990). However, some of these reasons may be ascribed to the fact that the endogenous auxin content of cuttings before severance varies with species. Endogenous auxin contents may be above or below threshold values for rooting, such that exogenous application can enhance or inhibit rooting to different degrees or have no effect on the cuttings. One possible role of auxin in rooting is in mobilizing carbohydrate reserves by enhancing the activity of hydrolytic enzymes (Nanda, *et al.*, 1968; Rana *et al.*, 1977). Middleton, Jarvis and Booth (1980) suggested that IBA enhances substrate concentration in the leaves of fresh cuttings thereby promoting the synthesis of endogenous auxin, which is essential in root initiation in cuttings. Haissig (1986) suggested that the role of auxin in increasing stem respiration rate in cuttings has a promotive effect on rooting. Auxin may also conjugate toxic compounds and reduce or inhibit the synthesis of rooting inhibitors (Haissig, 1988; Salisbury and Ross, 1978), such as gibberellin (Hartmann and Kester, 1983). Auxin can play a promotive role in the synthesis of enzymes and amino acid compounds as precursors in the eventual synthesis of Ribonucleic acid and Deoxyribonucleic acid (Salisbury and Ross, 1978), which carry coded genetic information during somatic cell division in the rooting process of cuttings. IBA has been found to enhance the movement of nitrogen, phosphorous and potassium to the rooting zone to promote rooting in cuttings (Haissig, 1986).

Leaf area

Leaf retention on cuttings exerts a strong stimulatory influence on root initiation (Hartmann and Kester, 1983; Reuveni and Raviv, 1981; Breen and Muraoka, 1974).

The presence of leaves on the cutting enhances adventitious root formation (Staden and Harty, 1988; Reuveni Raviv, 1981). Leaves on a cutting after severance promote rooting by producing endogenous auxin and enzymes, and enhancing the uptake of supplied auxin (Jarvis, 1986; Haissig, 1986; Breen and Muraoka, 1974). Leaves can also enhance rooting in cuttings by increasing the uptake of carbon dioxide, absorbing light energy, photosynthesizing carbohydrate, and with the aid of auxin, transporting all this to the base of the cuttings where energy is utilized from the carbohydrate for metabolism associated with root formation (Breen and Muraoka, 1974; Salisbury and Ross, 1978; Haissig, 1986 Jarvis, 1986). This is in some cases supplemented by the activities of newly developed buds and young leaves after budbreak.

In the present study, the rooting percentage of *R. heudelotii* was considerably enhanced by increasing leaf area and none of the leafless cuttings rooted. Rooting percentages differed significantly ($P < 0.05$) between the four leafed treatments. The rooting percentage exhibited an increasing trend with increasing leaf area. The highest rooting percentage of 90% was associated with the highest leaf area of 80 cm^2, while the lowest rooting percentage of 73% was recorded in the lowest leaf area of 12.5 cm^2. Similarly, the percentage of callused and sprouted cuttings, as well as the mean number of roots per rooted cutting each exhibited a positive relationship with increasing leaf area.

In the experiment involving *Irvingia gabonensis*, rooting percentage was found to be significantly different ($P < 0.001$) between the five treatments. The leafless cuttings all died. The proportion of rooted, callused and sprouted cuttings was observed to increase with increasing leaf area. Similarly, the mean number of roots per rooted cutting increased with increasing leaf area.

In the experiment investigating the effect of different leaf areas on rooting of *Gnetum africanum*, there were significant differences ($P < 0.001$) in rooting percentage between the five treatments. None of the leafless cuttings rooted and all died. The proportion of rooted and callused cuttings increased with increasing leaf area. Similarly, the mean number of roots per rooted cutting increased with increasing leaf area.

The fact that none of the leafless cuttings from the three species in these experiments rooted underlines the importance of leaves in the rooting of leafy stem cuttings of these species. Other workers have found the same result: Newton *et al.* (1992b), found that in a leaf area experiment with the cuttings of *Terminalia spinosa*, all the leafless cuttings failed to root. Breen and Muraoka (1974) also reported the failure of defoliated cuttings of a plum cultivar (marianna 2624) to root even when treated with IBA, while control cuttings rooted with only half the leaves present. However, Leakey *et al.* (1982b) reported 9% rooting success of leafless cuttings of *Triplochiton scleroxylon,* and 2% of *Hopea odorata* leafless cuttings have also been found to produce roots (Hamzah, 1992).

The fact that the rooting percentage of the three species, although *G. africanum* did not increase as much as the other two beyond 50 cm^2 each increased with increasing leaf area suggests that these species can root even with leaf areas larger than 80 cm^2. This contradicts the results of *Triplochiton scleroxylon* (Leakey *et al.*, 1982b) and *Khaya ivorensis* (Asanga, 1989; Tchoundjeu, 1989), where cuttings tended to maximize root percentage at leaf areas of 50 cm^2 and 10-30 cm^2 respectively, and *Terminalia spinosa* (Newton *et al.*, 1992b) and such other tropical species as *Nauclea diderrichii* (Leakey, 1990), which displayed no tendency towards an optimum leaf area. However, the concept of trimming the leaves of cuttings during propagation is to reduce water loss through excessive transpiration associated with large leave areas, with a view to obtaining an optimum leaf area per cutting, where the balance between water loss and photosynthesis is maximized (Leakey and Coutts, 1989) (see discussion in Chapter 3). Transpiration rates are, however, influenced by stomatal density, surface of distribution (on the upper or lower surface or both) of the leaf

(Salisbury and Ross, 1978). It is likely, therefore, that in some plants, large leaves may have fewer stomates and need the retention of very large leaf areas on their leafy stem cuttings for maximum rooting during propagation, while in some, the reverse may be true. The former situation might have been the case with the species investigated in this study.

The mean number of roots per rooted cutting in each of the three species increased with increasing leaf area in accordance with the observations of Breen and Muraoka (1974).

It is interesting to observe that the response of the three species to the increasing leaf area was pronounced. This may be ascribed to the fact that the retained leaves were able to photosynthesize while in the rooting medium (Leakey and Coutts, 1989; Newton *et al.*, 1992b), thereby supplying current assimilate to the basal rooting zone of the cutting.

Practical implications

In general terms, the results from these experiments have demonstrated that *Irvingia gabonensis*, *Ricinodendron heudelotii* and *Gnetum africanum*, like many other tropical tree species (Leakey *et al.*, 1994), are amenable to the low-technology vegetative propagation techniques developed for use in the tropics (Leakey *et al.*, 1992; Newton *et al.*, 1992a).

The three species in this study varied in their auxin requirements, with an optimum range of 8 - 40 μg for *R. heudelotii*, 150 - 200 μg for *I. gabonensis* and above 250 μg IBA for *G. africanum*. It would seem reasonable to work within these ranges for the two former species and to determine an appropriate dose for the latter species, by testing a wider range of concentrations. The droplet application method used in this study was found to be useful for experimental purposes, but would have to be developed into simpler application techniques (such as the 'quick dip' method) if propagation were to be carried out on a significant scale.

The three species rooted successfully in sawdust and, except for *G. africanum*, which produced the highest mean number of roots per rooted cutting in fine sand, all the species in this study produced the highest mean number of roots per rooted cutting in sawdust as well highest rooting percentage. Since sawdust is available at the project site and cost very little, this medium is strongly recommended for rooting of these species.

In this study, the cuttings of the three species displayed an increasing rooting ability, both in terms of the proportion of rooted, callused and sprouted cuttings and the mean number of roots per rooted cuttings, with increasing leaf area up 80 cm^2. In subsequent propagation work with these species, it will be worth investigating the leaf area that will maximize rooting to retain on the cuttings of these species for large scale vegetative propagation.

Suggestions for further work

The work described in this thesis is the start of a larger project about domestication, mainly of tropical plant species. Indeed, there are many species that deserve study. A few others, on which work has started include *Piper guianensis, Heisteria parvifolia, Passiflora foetida, Psidium guajava, Delonix regia*, coffee and *Leucaena leucocefala*. In all the multipurpose species for which there is great demand by the rural masses, in particular, both in the high forest and in savannah regions of Cameroon, there is an urgent need for genetic exploration and germplasm collection to be undertaken throughout their native ranges. This will involve identification of trees with superior genotype in terms of fruit/seed size, taste, (drawability in the case of bush mango), fruiting frequency, early/late fruiting, fruiting age and female:male ratio in the case of *Garcinia kola, Ricinodendron heudelotii* and *Gnetum africanum*, all of which are dioecious. This identification will usefully be done in many countries in West Africa where these plant species thrive naturally or are introduced, in order to cover their ecological range. After the identification phase, selection and collection of plant material in the form of cuttings, seeds, scions, microtissues (for culture), which constitute the germplasm collection will be needed. Material will be raised asexually by vegetative propagation techniques and some by seed in order to better quantify and appreciate genetic gains associated with cloning as against breeding and raising plant material from seeds. It would also be useful to establish gene banks by way of arboreta provenances (covering a wider area) and orchards or herboreta from where to further select in the future. It would also be useful to carry out some amount of socio-economic survey to determine the extent to which people rely on these multipurpose species outside the area and country where this study was carried out. It is intended that the results of the present investigation will be implemented in future projects in Cameroon to include both genetic conservation and the development of agroforestry systems, incorporating material of mature fruit tree species.

The main source of plant material for most of the propagation work in this study was seedling and sapling stockplants. In the case of *Ricinodendron heudelotii*, which is dioecious, it would have been useful if cuttings had been derived from stockplants of known sex.

It would also be useful to study the reproductively mature trees for phenology, fruit size, fruiting frequency, mast years, fruiting age and the kinds of animals which eat the fruits. In this way one would be better able to plan and time one's operations, so as to work at the right time of year, and take cuttings that will root and from the stockplants that meet one's phenotypic requirements.

Stockplant management is a very delicate operation, particularly in the tropics, where the growth of shoots can be very sporadic and vigorous, and can sometimes be difficult to manage. In this regard, it would be more useful to conduct many more light and fertilizer experiments on stockplants than two (Chapter 6). In this way, one would have a better understanding of the nutrient and light requirements of these stockplants for maximizing the subsequent rooting of cuttings taken from them. It would be necessary to study many more stockplant factors affecting the subsequent rooting of cuttings taken from them. Such other factors which this author could not study because of lack of means, include temperature, relative water content, carbohydrate content, vapour pressure deficit, stomatal conductance, rate of carbon dioxide uptake, variation in the carbohydrate contents of shoots of the same chronological age at different heights of the same plant, between plants of the same clone and between different clones. This is particularly important for fruit trees and multipurpose trees in general since the interest is in the fruits and other products which are non-timber as against timber where tree form is important, as a source of cuttings for large scale propagation programme with a view to bypassing the juvenile phase common in trees raised from seeds.

Another aspect which requires further study, is the relationship between cutting mortality and rooting percentage. It would be useful to analyse the causes of cutting death, and to differentiate between cuttings which did not root because they died, and those that had not rooted but were still alive at the end of experiments. At the current time, the causes of cutting death are not clearly understood, and the interpretation of symptoms of physiological "stress" is often difficult.

During the short time available to do the work reported in here, financial constraints, administrative bottlenecks and transport problems in particular made it difficult for this author to investigate most of the other factors that influence the rooting of leafy stem cuttings. It will be important to research these factors more particularly as they affect the rooting of the cuttings of multipurpose tropical plant species on which very little work in the area of vegetative propagation has been done to date.

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APPENDIX

Table A1 Analysis of variance of percentage rooting of leafy stem cuttings of *Ricinodendron heudelotii* stet to root in six different media, after five weeks in a non-mist propagator. Data were collected from 360 cuttings, 60 in each medium.

Source	DF	Sum of Squares	Mean Square	F Value	P
Treatment	5	1345.999	269.199	5.550	0.0029
Residual	18	872.778	48.487		
Total	23	2218.777			

Table A2 Analysis of deviance for stepwise regression to determine influence of media, block, node position and cutting length on the rooting ability of the leafy stem cuttings of Ricinodendron heudelotii set to root in six propagators, after five weeks in a non-mist propagator.

Source	d.f.	deviance	mean deviance	deviance ratio	P
+ block	1	0.336	0.336	0.27	NS
+ np	1	12.535	12.535	10.19	<0.01
+ treat	5	18.177	3.635	2.96	NS
+ sl	1	1.723	1.723	1.40	NS
Residual	332	408.353	1.230		
Total	340	441.124	1.297		

Table A3 Analysis of variance of percentage rooting of the leafy stem cuttings of *Ricinodendron heudelotii* treated with four different IBA concentrations, after four weeks in a non-mist propagator. Data were collected from 160 cuttings, 40 in each treatment.

Source	DF	Sum of Squares	Mean Square	F Value	P
Treatment	3	3166.880	1055.626	2.57	0.069
Residual	36	14782.524	410.625		
Total	39	17949.405			

Table A4 Analysis of deviance by stepwise regression to determine the influence of IBA concentration, node position, cutting length and cutting diameter on the rooting ability of the cuttings of *Ricinodendron heudelotii*, after four weeks in a non-mist propagator, treated with IBA.

Source	d.f.	deviance	mean deviance	deviance ratio	P
+ block	1	0.152	0.152	0.20	NS
+ sdb	1	12.653	12.653	16.23	<0.001
+ np	1	3.683	3.683	4.72	<0.05
+ treat	3	2.889	0.963	1.24	NS
Residual	130	101.355	0.779		
Total	136	120.734	0.887		

Table A5 Analysis of variance of percentage rooting of leafy stem cuttings of *Ricinodendron heudelotii* treated with four different leaf areas, after four weeks in a non-mist propagator. Data were collected from 208 cuttings, 52 in each treatment.

Source	DF	Sum of Squares	Mean Square	F Value	P
Treatment	3	50538.5469	16846.182	74.39	0.0001
Residual	48	10869.2491	226.442		
Total	51	61407.7960			

Table A6 Analysis of deviance for stepwise regression to determine influence of leaf area, node position, cutting length, cutting diameter and block on the rooting ability of the leafy stem cuttings of *Ricinodendron heudelotii*, after five weeks in a non-mist propagator, with leaves cut to different areas.

Source	d.f.	deviance	mean deviance	deviance ratio	P
+ block	1	0.258	0.2589	0.67	NS
+ treat	3	161.262	53.7540	139.38	<0.001
+ np	1	10.402	10.4022	26.97	<0.001
+ sdb	1	1.763	1.7638	4.57	<0.05
+ sl	1	1.137	1.1370	2.95	NS
Residual	184	70.960	0.3857		
Total	191	245.784	1.2858		

Table A7 Analysis of variance of percentage rooting of the leafy stem cuttings of *Irvingia gabonensis* set to root in six different media, after five weeks in a non-mist propagator. Data were collected from 330 cuttings, 55 in each medium.

Source	DF	Sum of Squares	Mean Square	F Value	P
Treatment	5	4398.798	879.759	13.13	0.0001
Residual	18	1205.841	66.991		
Total	23	5604.640			

Table A8 Analysis of deviance by stepwise regression to determine the effect of media, node position, cutting length and block on the rooting ability of the cuttings of *Irvingia gabonensis*, after five weeks in a non-mist propagator. Data was collected from 330 cuttings, 55 in each media treatment.

Source	d.f.	deviance	mean deviance	deviance ratio	P
+ block	1	3.0115	3.0115	3.52	NS
+ np	1	83.0077	83.0077	97.04	<0.001
+ treat	5	72.4650	14.4930	16.94	<0.001
+ sl	1	2.7961	2.7961	3.27	NS
Residual	321	274.5780	0.8554		
Total	329	435.8582	1.3248		

Table A9 Analysis of variance of percentage rooting of leafy stem cuttings of *Irvingia gabonensis* treated with five different IBA concentrations, after six weeks in a non-mist propagator. Data were collected from 250 cuttings, 50 in each treatment.

Source	DF	Sum of Squares	Mean Square	F Value	P
Treatment	4	3814.688	953.672	2.26	0.0775
Residual	45	18990.1831	422.004		
Total	49	22804.8717			

Table A10 Analysis of deviance for stepwise regression to determine the influence of IBA concentration, node position, cutting length, block and cutting diameter on the rooting ability of the leafy stem cuttings *Irvingia gabonensis* treated with IBA in a non-mist propagator, after six weeks.

Source	d.f.	deviance	mean deviance	deviance ratio	P
+ block	1	7.159	7.159	5.45	<0.05
+ treat	4	13.271	3.318	2.53	NS
+ np	1	6.962	6.962	5.30	<0.05
Residual	243	319.181	1.314		
Total	249	346.574	1.392		

Table A11 Analysis of variance of percentage of leafy stem cuttings of *Irvingia gabonensis* treated with five different leaf areas and set to root in a non-mist propagator, after five weeks. Data were collected from 275 cuttings, 55 in each treatment level.

Source	DF	Sum of Squares	Mean Square	F Value	P
Treatment	4	38272.9692	9568.2424	70.577	0.0001
Residual	50	6779.1711	135.5834		
Total	54	45052.1407			

Table A12 Analysis of deviance for stepwise regression to determine the effect of leaf area, node position, cutting length and block on the rooting ability of *Irvingia gabonensis*, in a non-mist propagator, after five weeks.

Source	d.f.	deviance	mean deviance	deviance ratio	P
+ block	1	2.8662	2.8662	3.31	NS
+ treat	4	136.8354	34.2089	39.53	<0.001
+ np	1	5.6156	5.6156	6.49	<0.05
Residual	268	5.6156	5.6156		
Total	274	377.2614	1.3769		

Table A13 Analysis of variance of percentage rooting of leafy stem cuttings of *Gnetum africanum* set to root in six different rooting media, after eight weeks in a non-mist propagator. Data were collected from 336, 56 in each medium.

Source	DF	Sum of Squares	Mean Square	F Value	P
Treatment	5	1928.0064	385.6012	0.0478	0.0478
Residual	18	2468.4509	137.1361		
Total	23	4396.4573			

Table A14 Analysis of deviance by stepwise regression to determine the influence of media, cutting diameter, node position and block on the rooting ability of the leafy stem cuttings of *Gnetum africanum* in a non-mist propagator, after eight weeks.

Source	d.f.	deviance	mean deviance	deviance ratio	P
+ block	1	0.062	0.062	0.05	NS
+ treat	5	28.436	5.687	4.54	<0.05
+ sdb	1	14.522	14.522	11.58	<0.001
Residual	328	411.269	1.254		
Total	335	454.289	1.356		

Table A15 Analysis of variance of percentage rooting of leafy stem cuttings of *Gnetum africanum* treated with five different IBA concentrations, after five weeks in a non-mist propagator. Data were collected from 275 cuttings, 55 in each treatment.

Source	DF	Sum of Squares	Mean Square	F Value	P
Treatment	43	1972.6547	493.1636	1.09	0.3702
Residual	50	22560.5693	451.2113		
Total	54	24533.2241			

Table A16 Analysis of deviance for stepwise regression to determine the effect of IBA concentration, block, node position, cutting diameter and cutting length on the rooting ability of the leafy stem cuttings of *Gnetum africanum* in a non-mist propagator, after five weeks .

Source	d.f.	deviance	mean deviance	deviance ratio	P
+ block	1	0.904	0.904	0.68	NS
+ sdb	1	3.172	3.172	2.40	NS
+ treat	4	6.841	1.710	1.29	NS
Residual	267	352.873	1.322		
Total	273	363.789	1.333		

Table A17 Analysis of variance of percentage rooting of leafy stem cuttings of *Gnetum africanum* treated with five different leaf areas and set to root in a non-mist propagator, for six weeks. Data were collected from 250 cuttings, 50 in each treatment.

Source	DF	Sum of Squares	Mean Square	F Value	P
Treatment	4	39713.1087	9928.277	26.98	0.0001
Residual	45	16558.7036	367.971		
Total	49	56271.8124			

Table A18 Analysis of deviance for stepwise regression to determine the influence of leaf area, block, node position, cutting length and cutting diameter on the rooting ability of the leafy stem cuttings of ***Gnetum africanum***, after six weeks, set to root in a non-mist propagator.

Source	d.f.	deviance	mean deviance	deviance ratio	P
+ block	1	1.8953	1.8953	2.19	NS
+ treat	4	128.7063	32.1766	37.22	<0.001
+ np	1	2.0604	2.0604	2.38	NS
+ sl	1	1.1215	1.1215	1.30	NS
Residual	242	209.1814	0.8644		
Total	249	342.9649	1.3774		

Table A19 Analysis of variance of percentage rooting of leafy stem cuttings from different stump heights of *Ricinodendron* heudelotii stockplants, after four weeks in a non-mist propagator. Data were collected from 240 cuttings, 80 in each treatment level.

Source	DF	Sum of Squares	Mean Square	F Value	P
Treatment	2	646.7261	323.363	23.66	0.0003
Residual	9	123.0278	13.669		
Total	11	769.7540			

Table A20 Analysis of deviance for stepwise regression to determine the influence of different stump heights, block, clone, cutting length and cutting diameter on the rooting ability of the leafy stem cuttings of *Ricinodendron heudelotii*, after four weeks in a non-mist propagator.

Source	d.f.	deviance	mean deviance	deviance ratio	P
+ block	1	0.000	0.000	0.000	NS
+ treat	2	16.187	8.094	6.35	<0.05
+ sdb	1	9.133	9.133	7.17	<0.01
+ clone	3	4.348	1.449	1.14	NS
+ sl	1	1.657	1.657	1.30	NS
Residual	230	292.930	1.274		
Total	238	4324.256	1.362		

Table A21 Analysis of variance of percentage rooting of leafy stem cuttings from shaded and non-shaded stockplants of *Ricinodendron heudelotii* in a non-mist propagator, after four weeks. Data were collected from 160 cuttings, 80 in each treatment.

Source	DF	Sum of Squares	Mean Square	F Value	P
Treatment	1	508.4638	508.463	2.82	0.1439
Residual	6	1080.2208	180.036		
Total	7	1588.6846			

Table A22 Analysis of deviance for stepwise regression to determine the influence of shading, block, clone, cutting diameter and length on the rooting ability of the leafy stem cuttings of *Ricinodendron heudelotii*, after four weeks in a non-mist propagator.

Source	d.f.	deviance	mean deviance	deviance ratio	P
+ block	1	6.208	6.208	5.85	<0.05
+ treat	1	12.288	12.288	11.58	<0.001
+ clone	4	4.991	1.248	1.118	NS
+ sdb	1	1.476	1.476	1.39	NS
Residual	152	161.281	1.061		
Total	159	186.244	1.171		

Table A23 Analysis of variance of percentage rooting of leafy stem cuttings from fertilized and non-fertilized stockplants of *Ricinodendron heudelotii*, after four weeks. Data were collected from 200 cuttings, 100 in each treatment level.

Source	DF	Sum of Squares	Mean Square	F Value	P
Treatment	1	438.351	438.351	11.05	0.0159
Residual	6	238.108	39.684		
Total	7	676.460			

Table A24 Analysis of deviance by stepwise regression to determine the influence of fertilizer (NPK) application, block, and cutting diameter on the rooting ability of the leafy stem cuttings of *Ricinodendron heudelotii*, after four weeks in a non-mist propagator.

Source	d.f.	deviance	mean deviance	deviance ratio	P
+ block	1	2.5161	2.5161	2.58	NS
+ treat	1	12.5292	12.5292	12.86	<0.001
+ sdb	1	5.2672	5.2672	5.41	<0.05
Residual	195	189.9524	0.9741		
Total	198	210.2648	1.0619		

Table A25 Analysis of variance of percentage rooting of leafy stem cuttings from stockplants of *Ricinodendron heudelotii* to different heights set to root in a non-mist propagator. Data were collected from 355 cuttings, 55 in each treatment.

Source	DF	Sum of Squares	Mean Square	F Value	P
Treatment	5	1841.198	368.239	9.14	0.0002
Residual	18	725.110	40.283		
Total	23	2566.308			

Table A26 Analysis of deviance by stepwise regression to determine the influence of pollard heights, block, cutting diameter, cutting length and node position on the rooting ability of the leafy stem cuttings of *Ricinodendron heudelotii.* after four weeks in a non-mist propagator.

Source	d.f.	deviance	mean deviance	deviance ratio	P
+ block	1	5.550	5.550	4.29	<0.05
+ sdb	1	8.737	8.737	6.76	<0.01
+ sl	1	5.664	5.664	4.38	<0.05
+ treat	5	8.508	1.702	1.32	NS
Residual	342	441.980	1.292		
Total	350	470.439	1.344		

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