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### Major heretofore intractable biotic constraints to African food security that may be amenable to novel biotechnological solutions

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#### **Abstract**

The input costs of pesticides to control biotic constraints are often prohibitive to the subsistence farmers of Africa and seed based solutions to biotic stresses are more appropriate. Plant breeding has been highly successful in dealing with many pest problems in Africa, especially diseases, but is limited to the genes available within the crop genome. Years of breeding and studying cultural practices have not always been successful in alleviating many problems that biotechnology may be able to solve. We pinpoint the major intractable regional problems as: (1) weeds: parasitic weeds (*Striga* and *Orobanche* spp.) throughout Africa; grass weeds of wheat (*Bromus* and *Lolium*) intractable to herbicides in North Africa; (2) insect and diseases: stem borers and post-harvest grain weevils in sub-Saharan Africa; *Bemesia tabaci* (white fly) as the vector of the tomato leaf curl virus complex on vegetable crops in North Africa; and (3) the mycotoxins: fumonisins and aflatoxins in stored grains. Abiotic stresses may exacerbate many of these problems, and biotechnological alleviations of abiotic stress could partially allay some predicaments. Some of these constraints are already under study using biotechnological procedures, but others may require longer-term research and development to alleviate the problems. Despite the huge impacts of post-harvest weevils and of mycotoxins in grains, these issues had not been given high priority in national biotechnological programs, possibly due to a lack of knowledge of their immensity. The need for public sector involvement is accentuated for cases where immediate profits are not perceived (e.g. lowering mycotoxin levels in farmer utilized grain, which does not increase yield) but where the public weal will gain, and will be invaluable, especially where the private sector supplies genes already isolated.

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### 1. Introduction

African agricultural productivity is severely limited by a large number of constraints that are biotic (arthropods, nematodes, diseases, weeds, rodents, birds); abiotic (drought, soil fertility, mineral toxicity); and infrastructural (low price to farmers and inability to

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compete with subsidized imports, high price of pesticide and fertilizer inputs, poor transportation and storage systems). This review will not deal with the infrastructure problems, but their solution is a key part of utilizing biotechnological solutions for biotic and abiotic stresses.

### 1.1. The major staple crops of Africa

The African continent is divided by the Sahara Desert into zones that are quite different in the primary crops

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Table 1
The major staple crops of northern and sub-Saharan (SS) Africa

Area	Crop	Area (Mha)	Yield (tons/ha)	Production (Mtons)
N. Africa <sup>a</sup>	Barley	2.8	0.8	2.4
SS Africa		0.9	1.0	9.6
World		54.0	2.4	131.6
SS Africa	Beans <sup>b</sup>	3.4	0.7	2.3
World		24.7	0.7	17.9
SS Africa	Cassava	10.9	8.9	97.0
World		16.9	10.6	180.1
SS Africa	Cowpeas	8.8	0.4	3.1
World		9.1	0.4	3.7
N. Africa	Maize	1.3	5.3°	7.0
SS Africa		21.3	1.3	27.0
World		138.9	4.3	602.0
SS Africa	Millet	21.0	0.6	14.4
World		36.9	0.7	42.1
N. Africa	Sorghum	0.3	5.0°	0.9
SS Africa		22.5	0.8	19.9
World		42.1	1.3	55.3
N. Africa	Tomatoes	0.3	30.0	9.1
SS Africa		0.3	7.4	2.3
World		4.0	27.0	108.0
N. Africa	Fresh vegetables	0.01	13.1	1.2
SS Africa		1.7	6.2	10.5
World		15.8	14.7	233.2
W. Africa	Wheat	1.7	1.8	12.0
SS Africa		5.9	1.6	2.6
World		210.8	2.7	568.1

Compiled from FAO Stat for 2002.

Cassava and bananas are major local crops in some countries, but are excluded from further discussion due to their localized nature.

cultivated, with the northern zone mainly dependent on wheat and barley as grain crops, grain legumes as the primary protein source, and a high dependency on irrigated fresh vegetables in the diet (Table 1). In sub-Saharan Africa (SSA) the majority of calories come from grains other than wheat (sorghum, maize, and the millets) along with/or cassava, with less protein supplementation from grain legumes (mainly cowpeas and beans) and a more limited array and amount of fresh vegetables. Two possibly interrelated areas differentiate the 80% agrarian societies of Africa from the developed world; a life expectancy more than 20 years lower than the developed world, with a much higher infant mortality; and grain yields about a third of the world averages. This review is aimed at pinpointing the major biotic constraints to subsistence agriculture in Africa that have not been adequately overcome by conventional technologies, and then to assess whether the constraints may be overcome by using various biotechnological tools.

### 1.2. Where conventional technologies are inadequate

Most of the staple crops of Africa are introduced, except for sorghum and millets that are often no longer purely African. Some sorghum and millet varieties contain foreign genetic input. The crops have been heavily selected by farmers and bred by scientists to overcome indigenous diseases and insects not found elsewhere. The continual successes in dealing with recently evolved strains of fungal and viral pathogens are of great credit to plant breeders. Still, there are cases where breeders cannot keep up, or where there is no inherent resistance within the crop, especially with some viral diseases. In addition, endophytic fungal infections appeared that hardly reduce yields but produced toxins, which have largely been ignored except for export crops. The low chronic levels of toxins in farmers' diets have not been addressed. Expensive fungicides could be used for export crops, but the lack of recognition of the problem together with the prohibitive expense of fungicides has allowed the mycotoxin problems to proliferate.

Insect pest infestations have either been "accepted" because insecticides are too expensive, or treated with low amounts of insecticides, often applied using methods that would be deemed unsafe and unacceptable in the developed world, i.e. in contravention of the pesticide label. There are also cases where insecticide resistance has evolved.

Weeding is predominantly a backbreaking task for women, often consuming 80% of their waking hours (Akobundu, 1991). Weed issues have been largely ignored both on the farm and in the research community, possibly due to an element of male chauvinism. Hand weeding has created a niche for weeds that could not be hand weeded, and which could not be directly killed by herbicides, without killing the crop. Where herbicides have been used, weeds have evolved resistance, or new weed species have appeared that could not be selectively controlled by herbicides.

There is a necessity to ascertain whether biotechnologies can supply rapid, safe, cost-effective solutions to the intractable biotic constraints. The abiotic constraints of low fertility soils, acidity with concomitant mineral toxicities as well as transient drought, while amenable to biotechnological intervention, are beyond the scope of this review. The institutional and infrastructure constraints to African agriculture are amenable to positive human intervention, and could facilitate rapid adoption of the yield and quality enhancing biotechnologies described below. The rapid adoption of hybrid maize in much of eastern Africa and Bt cotton in South Africa (James, 2002) through marketing efforts of the private sector are cases in point.

<sup>&</sup>lt;sup>a</sup> Includes the Maghreb countries and Egypt.

<sup>&</sup>lt;sup>b</sup>Does not include faba beans.

<sup>&</sup>lt;sup>c</sup>The average yield is high due to irrigation in key areas.

### 1.3. The value of biotechnology to subsistence agriculture

It is often stated that modern biotechnology has little to offer poor subsistence farmers, just having value for large-scale farmers. The actual data contradict that contention. Farmers prefer inputs that are within or on seed. That is why subsistence farmers purchase certified or commercial seeds of open pollinated varieties every 3–7 years. The use of hybrid maize seed is becoming more widespread throughout Africa because of the recognized hybrid vigor and increased yield. African farmers commonly use inexpensive seed dressings of insecticide and/or fungicide, but inherent disease resistance of new varieties is invariably chosen over fungicides.

Bt cotton genetically engineered to be resistant to insect pests was more rapidly adopted by small farmers in South Africa than by large-scale farmers (Bennett et al., 2003). The small-scale farmers prefer having insect resistance in the seed instead of having to spray insecticides. This African experience parallels that with Bt cotton adoption in China and India (James, 2002). Thus, biotechnology is clearly amenable to small-scale subsistence farmers in Africa, in all the major constraint case studies cited below.

### 2. Methods

The authors wished to ascertain, quantify, and prioritize the constraints to food production and security in Africa that are not being adequately addressed by traditional agricultural research and development. Time and financial constraints precluded performing a large scale, multi-year, grid survey. Instead a three level, subjective study was performed as described below. The study does not include a direct farmer participatory element because it was clear from preliminary estimations that farmers do not recognize all constraints, as some are insidious (see Section 3.6 below). Instead, African scientific experts who continually meet with farmers and see their problems were chosen, as described below. They performed the estimates with the assistance of colleagues throughout the region. Their results were presented at a United Nations Industrial Development Organization (UNIDO) meeting and were crosschecked during and after the meeting with biotechnologists from countries throughout the region, who outlined their governments' priorities for biotechnology.

### 2.1. Delphic choice of experts

Delphic methods (Khorramashahgol and Moustakis, 1988) of obtaining expert input and pooling information to achieve a consensus view were used. A wide ranging

group of western and African scientists were asked to name and evaluate leading African scientists dealing with agricultural constraints, particularly scientists who had close dealings with farmers yet had many regional contacts with similar scientists. A committee then chose scientists from within the top rankings, while also ensuring a broad regional distribution.

# 2.2. Subjective analysis of intractable constraints and estimation of damage and cost

The chosen scientists were asked to first obtain subjective consensus on up to two major regional constraints in their area of expertise. A proviso was that the major constraints chosen had not been amenable to conventional solutions. They were then to provide the econometric estimates provided herein in uniform format, all in consultation with colleagues of their choice throughout the region.

### 2.3. Partial cross-corroboration via national biotechnology priorities

The experts (the African authors of this article) presented their conclusions for cross-corroboration to a group of international and African biotechnologists and government officials dealing in agriculture. The other delegates to this meeting presented national biotechnology program priorities that invariably included most but not all of the constraints discussed below among their top ranking priorities, along with more localized priorities in crops not grown as widely throughout the region (e.g. bananas, plantains, cassava). The national program reports were typically primarily crop oriented, and the constraints listed within the crops, and the national rankings were derived without numerical estimates of damage.

### 3. Results and discussion

# 3.1. Grass weed constraints (Lolium rigidum and Bromus spp.) in northern Africa

Yield losses due to weeds in cereal crops have been estimated to reach 20% in Tunisia (B. Kalbous, personal communication), 20% in Algeria (FAO, 2000) and 30% in Morocco (Zimdahl and El Brahli, 1992). The weed flora associated with cereals is highly diversified and the major weed species include *Bromus* spp., *Avena sterilis*, *Phalaris* spp., *Lolium* spp., *Solanum eleagnifolium*, *Raphanus raphanistrum*, *Ridolphia segetum*, and *Cynodon dactylon*.

The genus *Bromus* is the most widespread weed group in cereal growing regions in northern Africa. The *Bromus* spp. are winter annual grass weeds that

germinate in the fall, often simultaneously with planted winter wheat, resulting in significant yield losses. *Bromus rubens*, *B. madritensis*, *B. sterilis*, and *B. rigidus* are the four most common bromegrass species, with *B. rigidus* being the major species that causes problems for cereal growers in the region. Factors that contribute to the development and spread of bromegrasses in cereals include monoculture, minimum tillage, the intensive use of herbicides that control other weeds but not the brome grasses, and the lack of effective and selective herbicide to control the bromes in wheat (Hamal et al., 2001; Souissi et al., 2000).

In Morocco, B. rigidus infests 30% of the total cereal area and reduces wheat yields up to 15% (Table 2). In field surveys conducted in the Saiss area, where brome grass causes serious problems in wheat, B. rigidus was the most dominant species, occurring in 47% of the surveyed fields with 40% coverage (Hamal et al., 2001). Heavy infestation of cereals with *Bromus* spp. has resulted in estimates of yield losses of 66-98% in Sais (Hasnaoui, 1994), and 43% in Taleb (Hamal et al., 1998). In Tunisia, great brome (B. diandrus syn. B. rigidus subsp. gunosoi) has become a serious weed in cereal growing areas and has caused considerable reductions in wheat yields. The infested area was estimated to be 12,000 ha in 1998 in the northern region and is increasing (Souissi et al., 2000). The weed normally reduces wheat yield by 20-50% and up to 80% in heavily infested areas, costing growers up to \$1.6 million/year (Table 2). In Algeria, B. rigidus infests about 500,000 ha of the area cropped with cereals. Surveys conducted in 359 cereal fields in the Constantine high plains in the northeast of Algeria have shown that Bromus rigidus and Avena sterilis were the most frequent and abundant grass weeds in cereals (Fenni et al., 2001). Yield losses due to weeds in cereals including brome grass have been estimated at 25–50% (Table 2).

Further complicating the fact that weed control is poor in cereals, the evolution of herbicide resistance is becoming a larger problem in Tunisia. The first report of evolved herbicide resistance in Tunisia concerned *Lolium rigidum* (ryegrass) in cereals in 1996 (Heap, 2003). *L. rigidum* is widespread in cereal growing regions of the country. Most farmers rely on acetyl-CoA carboxylase (ACCase)-inhibiting herbicides for control of *Lolium*, as these grass herbicides are highly effective

for post-emergence control of grasses in cereals and broadleaf crops. The farmers had weed control failure and were not able to achieve control with alternative herbicides. The weed has evolved resistance to all ACCase inhibitors tested, e.g. diclofop methyl and others (Gasquez, 2000), and there are no other herbicides that will control this weed. The total infested area with herbicide-resistant *Lolium* has been estimated to be 4000–40,000 ha and is increasing.

### 3.1.1. Conventional technologies that have failed

Hand pulling, tillage and to a lesser extent herbicides and crop rotation are the commonly used methods to control weeds, including bromegrasses in cereals in the Maghreb countries. Although prevention is the most basic of all management strategies, it is very weakly adopted and in most cases neglected by farmers.

Despite the use of herbicides by farmers to control bromegrasses in cereals and broadleaf crops, their efficacy is limited and depends on factors that include the time of application, the growth stage of the weed, crop cultivars and moisture. Crop rotations, when well managed, can greatly reduce the weed infestation. However, although bromegrass populations can be reduced by the use of post-emergence herbicides in legumes, farmers are giving up growing legumes in rotation with cereals because of the absence of motivating price policies and inadequate marketing systems for grain legumes. Cereal monoculture using the same management practices year after year has become the predominant cropping system, resulting in extensive herbicide resistance.

### 3.1.2. Potential biotechnological solutions and status

3.1.2.1. Biocontrol and allelochemicals. Much interest has developed in exploiting fungal and bacterial plant pathogens for biological control of weeds, as reviewed and assessed by Evans (2002). The potential of deleterious non-parasitic rhizosphere bacteria that aggressively colonize roots has been demonstrated for biological control of seedlings of important weeds (Kremer and Kennedy, 1996; Souissi and Kremer, 1994). For instance, deleterious rhizobacteria have been used to stunt brome growth in wheat fields in the northwestern USA. Kennedy et al. (1991) have identified *Pseudomonas* spp. with potential as effective candidates

Table 2 Effects of *Bromus* species on wheat in the Mahgreb

	Morocco	Tunisia	Algeria
Area infested (1000 ha) Yield reduction Value of yield losses	1440–1650 15% (98% HIA)	12 Up to 50% (80% HIA) \$1.6 million (durum wheat)	500 Up to 50% \$25–50 million (all weeds)

HIA: Highly infested areas

Source: compiled by T. Souissi with damage data kindly provided by Dr. K. Saffour (INA, Morocco) and Dr. H. Abdelkrim (INA, Algeria).

to suppress Bromus tectorum (downy brome) in winter wheat. The application of the deleterious rhizobacteria has increased wheat yield 18-35% by reducing bromegrass populations in field tests (Kennedy et al., 1991). The deleterious activity of the rhizobacteria is attributed to the production of a phytotoxin that is readily absorbed by the weed (Gurusiddaiah et al., 1994). Both too great a host specificity and insufficient efficacy of deleterious rhizobacteria are major considerations that have limited their adoption in the USA. For example, some are extremely host-specific and target only one weed when several may be interfering in the crop; others only suppress weed growth without completely killing the weed (Kremer and Kennedy, 1996). Similar work with selected rhizobacteria for growth suppression of B. diandrus is being conducted in Tunisia. Early screening has identified deleterious rhizobacteria that reduce brome growth by more than 40% and stimulate wheat growth by 43% in pots (T. Souissi, unpublished data). These promising results emphasize the need for more research efforts in the area of biocontrol of weeds within the region, especially for those weeds that are difficult to control with available herbicides. However, funds are lacking, and insufficient development and risk assessment infrastructures will be major constraints to the adoption of bioherbicides in weed management programs in the region.

Despite considerable research conducted on biocontrol of weeds with plant pathogens, only six agents are registered as bioherbicides in the USA, Canada, Japan and South Africa (Charudattan and Dinoor, 2000). This small number can be attributed to several constraints, including limited efficacy, economics of unprofitable markets, technological difficulties in mass production and formulation as well as not being as cost-effective as herbicides (Charudattan, 1991). The efficacy of biocontrol agents could be enhanced with a variety of transgenes to enhance their virulence against grass weeds, their persistence in soil, and their selectivity between wheat and the grass weeds (Amsellem et al., 2002; Duke, 2003; Gressel, 2002; Vurro et al., 2001). Hence, more efforts are needed to characterize genes, e.g. for virulence and production of toxins, hormones and enzymes, which may improve the efficacy of the biocontrol agents. However, regulatory restrictions may render the practical use of genetically engineered pathogens difficult.

Wheat could also be engineered to produce allelochemicals that would either kill or suppress weeds growing nearby (Duke, 2003; Gressel, 2002). If this were done, the most efficient method of planting would be not to grow wheat in spaced rows, but to have the plants equally spaced (by broadcasting) to have more area covered by the allelochemicals. Such a return to ancient technologies provides for more efficient suppressive shading of weeds by wheat and barley.

3.1.2.2. Herbicide resistant wheat and barley. Virtually all presently used wheat selective herbicides (except those applied with a protectant) are detoxified by cytochrome P450s in wheat. Grass weeds contain similar cytochrome P450s, but at lower levels, and thus succumb to the herbicides, until they evolve higher levels of these enzymes, which some have done (Gressel, 1988). Some new herbicides contain a protectant that induces the elevation of herbicide-degrading glutathione transferases in wheat, but the grass weeds have the same enzymes and could easily evolve constitutively higher levels, and thus resistance.

The simplest biotechnology to achieve control of weeds such as the *Bromus* spp. that are naturally somewhat resistant to wheat herbicides, and Lolium that has evolved resistance to wheat herbicides, is to develop new herbicide resistant crops. The best new herbicide resistant wheats that could allow selective control are ones where the transgenes encode for genes not found in the grass weeds, e.g. genes from microorganisms (Gressel, 2002). Wheat has been engineered to have resistance to glyphosate and to glufosinate, both herbicides that will control grass weeds. Glyphosateresistant wheat is being released in North America for the purpose of inexpensive early post-emergence weed control, and will be especially good at controlling the herbicide resistant grass weeds that have evolved there. Glufosinate resistant transgenic wheat has been generated by many groups, using the bar gene from actinomycetes, but only as a genetic marker, and not for commercial purposes. The herbicide is inherently expensive to manufacture and is unlikely to be cost effective.

The close relatedness of wheat to its grassy weeds and their enzymatic similarity are major factors necessitating the development of herbicide resistant wheat bearing transgenes from alien species. The close relatedness is also the source of potential problems. In parts of North America Aegilops cylindrica is a major weed of wheat, and the two amphipolyploid species both contain an interchangeable D genome, which moves with ease between them (Wang et al., 2000), and could carry transgenes. Wheat can also have homeologous pairing in hybrids with some of its grassy relatives that do not have homologous chromosomes, including Ae. ovata, Ae. triuncialis and Ae. ventricosa, which are present in the Mediterranean basin. This allows movement of genes (including transgenes) between the non-homologous chromosomes.

Transgenic herbicide resistant cultivated barley (*Hordeum vulgare*) that would allow grass weed control bears the same risks; there is weedy *H. murinum* and many wild barleys (*H. maritimum* and *H. bulbosum*) that are also present in N. Africa (Bonnet and Barratte, 1896) that might possibly introgress the transgene conferring resistance.

Thus, because of the possibilities of introgression with related wild species, if the simple solutions of herbicideresistant wheat or barley are to be considered for northern Africa, they should be considered together with genetic containment failsafe mechanisms. The most widely discussed containment is to engineer the traits into the maternally inherited chloroplast genome (Daniell, 2002). Pollen can transmit chloroplast genome at frequencies of more than 0.03% (Wang et al., 2004), which is orders of magnitude greater than the nuclear mutation frequency for resistance. Additionally, the use of this procedure does not preclude crop-weed hybrids where the related weed is the pollen parent, and continues to be the pollen parent in backcrosses. Thus, containment failsafe mechanisms should be coupled with mitigation mechanisms, e.g. where the herbicide resistant transgene is tandemly coupled with traits that benefit the crop but are deleterious to a crop-weed hybrid and its backcross progeny (e.g. anti-shattering genes, dwarfing genes, etc.) (Al-Ahmad et al., 2004; Gressel, 1999).

### 3.2. Parasitic weed species constraints

Orobanche and Striga spp. are parasitic, higher flowering plants that each specifically parasitizes the roots of crop hosts following germination due to specific stimulants from the crop roots (Press and Graves, 1995). After attachment, they penetrate into the vascular system of the crop, removing water, photosynthates and minerals. The Orobanche species are holoparasitic, i.e. are missing part of the chloroplast genome (Thalouarn et al., 1994; Young et al., 1999) and perforce, are wholly dependent on the host for nutrition. The Striga spp. are hemi-parasitic, and can photosynthesize about 20% of their needs after emerging from the soil. The Striga spp. cause a chlorotic stunting ("bewitching") of grain crops at the whorl stage, hence the common name, witchweeds. The Orobanche spp. were initially found on broom plants (leguminous shrubs) and thus their descriptive common name of broomrapes. All these parasitic weeds set tens of thousands of tiny but long-lived seeds on each of the emerging flower stalks.

### 3.2.1. Orobanche spp. in northern Africa

In northern Africa, the economically important Orobanche species are O. crenata, O. foetida and O. ramosa. O. crenata and O. foetida mainly attack legumes, especially faba bean. O. ramosa is less important and infests tomato, tobacco, and potato. O. crenata is the most damaging and widespread Orobanche species in the food legume crops in northern Africa, with faba bean being the most seriously affected crop (Table 3). According to Parker (1986), the total faba bean infested area in the Mediterranean region was then

Table 3
Losses in faba beans from *Orobanche* spp.

Country	Area infested (ha)	% of total cropping area infested	% yield loss	Tons lost	Million \$ loss
Tunisia Morocco Egypt	5–10,000 133,000 <sup>b</sup>	45 <sup>a</sup> 50 20 <sup>f</sup>	50-80 12-38 <sup>c,d</sup> 5-33 <sup>g</sup>	70,000	16–24 <sup>e</sup> 10 <sup>f</sup>

*Orobanche* spp. infestation can result in total yield loss in Algeria. Compiled by T. Souissi with data provided by N. Zermane (University of Kassel, Germany) and K. Zaffour (INA, Morocco).

- <sup>a</sup> Zermane et al. (2001).
- <sup>b</sup> Aber (1984).
- <sup>c</sup>Schmitt (1981).
- <sup>d</sup>Geipert et al. (1996).
- e Betz (1999).
- <sup>f</sup>Hassenein and Salim (1999).
- g Sauerborn and Saxena (1986).

estimated to be about 800,000 ha of which about 20% were located in northern Africa including Algeria, Morocco and Tunisia. The situation has become much worse (Zermane, 2000). In Morocco, the total infested faba bean area was estimated at about 133,000 ha (about 50% of the total faba bean area) and the total area infested with O. crenata exceeds 160,000 ha when other infested crops such as lentil, peas and chickpeas are considered. The yield losses to faba beans were estimated at 12% (cf. Yazough and Klein, 1999), and 33% in some heavily infested districts (Geipert and Sauerborn, 1996). Estimates of 38% result in 70,000 tons of losses. According to Betz (1999) losses could reach US \$16 million over the whole country and amounted to about \$24 million per year when all food legumes are considered (Table 3).

In Tunisia, both *O. crenata* and *O. foetida* attack faba bean. Field surveys conducted during April–May 2000 in the major legume cropping areas in Tunisia showed that *Orobanche* was present in 45% of the surveyed faba bean fields (Zermane et al., 2001). The areas infested with both *O. crenata* and *O. foetida* have been estimated to vary from 5000 to 10,000 ha (Kharrat, personal communication). Losses in faba bean yield were estimated to range from 50% to 80% in fields with medium and high levels of infestations, respectively.

In Egypt, *O. crenata* is one of the major constraints to the production of faba bean, which is the most important food legume in the country. *O. crenata* occurs in 20% of the total area cropped with faba bean, of which half is considered to be highly infested (Table 3). Sauerborn and Saxena (1986) reported yield losses from 5% to 33% caused by *Orobanche* in faba bean in Egypt. Losses were estimated at about 20,000 tons of faba bean seeds, which cost about 10 million dollars (Hassanein, 1999).

Although *O. ramosa* is less important than *O. crenata*, it constitutes a real threat to Solanaceous crops in the region. Tomato, potato, and tobacco are among the crops parasitized by *O. ramosa*.

### 3.2.2. Striga spp. in sub-Saharan Africa

The genus *Striga* (Scrophulariaceae) contains a group of obligate hemi-parasitic flowering weeds that are major biotic constraints to cereal and legume production in SSA (Table 4). There are four agriculturally important species of this hemi-parasite in the family in the region: *Striga hermonthica*, which is the most economically significant species, spreading in western, eastern and central Africa; *S. asiatica* is economically significant only in eastern Africa and southern Africa; *S. forbesii* is significant only in niche areas in Zimbabwe; and *S. gesnerioides* is significant mainly on cowpeas in western and eastern Africa (e.g. Nigeria and Tanzania).

Striga spp. are native to the grasslands of the Old World tropics, reaching their greatest diversity in the region where they have co-evolved with the cereals, especially sorghum, millets and upland rice. They have spread widely, becoming a scourge to cereal production (including maize) and legumes (cowpeas) where fertility is low and water/rainfall is low or erratic. The estimated area of cereals infested with Striga (excluding maize) and cowpeas is presented in Table 5. The genus is most widespread in western Africa where it covers 64% (17 million hectares) of the cereal production area with a potential coverage of almost 100% in the semi-arid and sub-humid tropical zones. The coverage in eastern and central Africa is significantly lower at 23% (3 million hectares), and lowest in southern Africa with only 1.6 million hectares mostly in Mozambique. The countries with greatest Striga infestations on sorghum and millets are shown in Table 6. The highest infestations are in Nigeria (8.7 million ha), Niger (5.0 million ha), Mali (1.5 million ha) and Burkina Faso (1.3 million ha). These are potential disaster areas for crop losses, and together with Tanzania having only 650,000 ha infested, account for about 90% of total area. Both Nigeria and Tanzania (together with Kenya) have heavy Striga infestation in maize (Table 6).

The most severe *Striga* infestations have been found in older crop fields, to the extent that very old cereal

Table 4
Estimates of production losses due to *Striga* in sub-Sahara Africa

	Sorghum and millets*	Maize	All crops
Area affected (million ha)	21.9	4.33	26.23
Estimated yield loss (%)	26	40	33
Estimated loss in production (million ton)	8.60	2.07	10.67

<sup>\*</sup>Includes cowpeas in West Africa (summarized by A.B. Obilana).

fields with extreme erosion of fertility combined with water scarcity (a common situation in semi-arid tropics) are abandoned by farmers. Where options for field abandonment and farmer migration to newer fields are limited, crop rotations with catch crops or trap crops (e.g. sesame, soybeans, leguminous green fallow, and pearl millet where it is not a host) are practiced.

In many places in Africa, the Striga problem has reached epidemic proportions with the situation being worst in subsistence agriculture due to several factors. Yield losses from damage by Striga are often very significant (Table 7), ranging from 10% to 70%, depending on the crop cultivar, degree of infestation, rainfall pattern and soil degradation, and estimated at 40% on average (Elemo et al., 1995). The countries in the SSA with highest food production losses due to Striga with averages ranging from 20% to 90%, amounting to 8 million tons in 11 countries (6 of these in West Africa alone) are summarized in Table 7. The data in Tables 4–7 demonstrate the highly variable nature of *Striga* infestation in SSA—very erratic across infested fields, and highly diverse and differential across regions. This scenario is highlighted by absence of *Striga* on pearl millet in east central and southern Africa, while both these crops are severely infested in western Africa. The erratic infestation across fields and in different seasons and years also account for some of the difficulties in compiling the tables from national estimates and data on infestation. This is one area that needs further addressing.

One major reason for these losses relates to food grain and seed aid to farmers in these areas; seed or grain consignments from Striga infested fields are being distributed on a large scale, resulting in wider spread of the serious infestation. Twenty to 40% of commercial seed lots in some markets were contaminated with Striga (Berner et al., 1994). Another major reason is the lack of adoption of available control methods by resource poor farmers. Although several potential control measures have been developed in the past decades, most of these methods (including the use of chemical herbicides, nitrogen fertilization and soil fumigation) are too costly for poor subsistence farmers that make up about 75– 80% of farmers in SSA. Their plight has been compounded by the environmental and policy factors that fostered Striga spread.

### 3.2.3. Conventional technologies that have failed

Orobanche is mainly a problem on introduced crops with considerable resistance within the genomes of indigenous species such as melons in the Middle East, which have no yield loss despite multiple attachments of the parasite. Crop breeding has so far been of but little use in obtaining resistance in the crops introduced into Africa, despite years of effort. Evolution seems not to have provided genes for resistance to crops that did not

Table 5
Estimated area under *Striga* infestation in Africa (excluding maize)

Country	Area ('000	ha) cultivated	Present crop	yields (t/ha)	Striga infest	ed area (sorghum)	Est. % yield loss	Yield loss ('000 ton
	Sorghum	Pearl millet	Sorghum	Millet	'000 ha	% total	_	
(A) East and	central Afric	ea <sup>a</sup>						
Burundi	50	13	1.22	0.77	_	_	_	_
Eritrea	160	17	0.62	0.30	64	37.5	20-60	30-90
Ethiopia	1760	250	1.27	0.95	528	30.0	25	500
Kenya	150	86	1.05	0.42	80	53.3	35-40	50-60
Rwanda	80	2	1.05	0.83	1.6	2	5	5
Somalia	500	_	0.46	_	150	30	15	30
Sudan	6250	2500	0.66	0.25	1600	25.6	30	1060
Tanzania	690	320	0.50	0.71	650	90	Up to 90	550
	9640	3188			3074	32	22–38	2225–2295
(B) Southern	Africa <sup>a</sup>							
Uganda	270	410	1.50	1.57	27	10	10	<1
Botswana	100	6	0.11	0.17	30	30	25	8
Malawi	54	34	0.68	0.60	8	15	20	40
Mozambique	376	51	0.52	0.26	150	40	35	_
Namibia	53	233	0.38	0.28	_	_	_	_
South Africa	179	212	1.94	0.18	18	10	5	20
Swaziland	1	_	0.60	_	0.2	15	10	<1
Zambia	42	64	0.66	0.77	6	15	15	5
Zimbabwe	133	252	0.5	0.26	27	20	25	20
Total	1208	1262			266	22	18	95
(C) West Afri	ica <sup>b</sup>							
Benin	142	38	0.78	0.66	9	5	10	10
Burkina Faso	1398	1239	0.89	0.64	1319	50	35-40	710-820
Cameroon	497	54	0.75	1.01	55	10	15-20	70–90
Chad	550	591	0.71	0.48	114	10	15	100
Cote de'Ivore	50	84	0.60	0.84	7	5	5	5
Gambia	20	97	1.66	1.08	_	_	20-35	30-50
Ghana	311	202	0.91	0.83	77	15	35	170
Guinea	7	11	0.70	0.83	1	5	10	1
Mali	957	1205	0.77	0.60	1513	70	40	580
Niger	2261	4866	1.08	0.38	4989	70	40–50	930–1160
Nigeria	5700	5200	1.07	0.89	8720	80	35	3750
Senegal	133	895	0.87	0.61	411	40	20	120
Togo	184	130	0.77	0.52	6	2	35	70
Total	12,210	14,612		V.52	17,221	64	24–27	6555–6926

Compiled by A.B. Obilana.

co-evolve and did not co-exist with these parasitic weeds, except during the past millennium. One breeding effort more likely to succeed is where a few genes from different wild *Sorghum* species, each conferring a modicum of tolerance due to different genetic mechanisms, are combined in domestic sorghum. Different wild *Sorghum* species each have some resistance to less production of a *Striga* germination stimulant, others due to inhibition of *Striga* haustorium formation, and others to suppressed *Striga* growth after attachment. Conversely, one could argue that the genetic effort will be complicated by linkage problems with yield reducing and other undesirable genes in these wild species, and it

would be best to isolate the genes for transformation into elite varieties, not only of sorghum but of other crops as well.

3.2.3.1. Orobanche. Over the last decade, research activities on Orobanche control have been intensified in northern Africa, Spain and Israel. Still, there is no practical and economical means of Orobanche control available that can be easily adopted by farmers.

In general, preventive methods that reduce the dissemination of the parasitic weed are neglected by farmers. Cultural methods that include delayed planting, hand-pulling, use of trap and catch crops, as well as

<sup>&</sup>lt;sup>a</sup>On sorghum only in east central and southern Africa. Pearl millet is not infested by Striga in these regions.

<sup>&</sup>lt;sup>b</sup>Includes both sorghum and pearl millet combined in West Africa.

Table 6 Sub-Saharan Africa countries with most *Striga* incidence/infestation

Country	Sorghum area ('000 ha)	Millet <sup>a</sup> area ('000 ha)	Maize area ('000 ha)	Striga infest	ed area			
				Sorghum ar	Sorghum and Millet <sup>b</sup>		Maize	
				('000 ha)	% total	('000 ha)	% total	
Botswana	100	6	20	30	30	2	10	
Burkina Faso	1398	1239	261	1318	50	26	10	
Eritrea	160	17	3	64	40	0	0	
Ethiopia	1760	250	1606	528	30	80	5	
Kenya	150	86	1502	80	53	225	15	
Mali	957	1205	195	1513	70	20	10	
Mozambique	376	51	1221	150	40	122	10	
Niger	2261	4866	_	4989	70	_	_	
Nigeria	5700	5200	4111	8720	80	904	22	
Senegal	133	895	61	411	40	3	0.05	
Sudan	6250	2500	169	1875	30	17	10	
Tanzania	690	320	1785	650	90	214	12	
Total/mean	19,935	16,635	10,934	20,330	56	1613	15	

Compiled by A.B. Obilana from reports of A.B. Obilana, F. Kanampiu and D. Friesen.

Table 7
Sub-Saharan Africa countries with the highest food production losses due to *Striga*<sup>a</sup>

Country	Estimated % yield loss <sup>a</sup>	Yield loss ('000 tons)
Burkina Faso	35–40	710–820
Eritrea	20–60	30-90
Ghana	35	170
Kenya	35–40	50-60
Mali	40	580
Mozambique	35	40
Niger	40-50	930-1160
Nigeria	35	3750
Sudan	30	1230
Tanzania	up to 90	550
Togo	35	70
Total/mean	39–45	8110-8520

Compiled by A.B. Obilana, from NARS documents, reports and personal records.

crop rotation can be somewhat effective in controlling *Orobanche*, although they have limitations. For instance, in Tunisia, hand weeding is the main control measure used to control *Orobanche*. However, this is not effective in highly infested fields and is very expensive. Kharrat and Halila (1996) reported that continuous hand pulling of *Orobanche* in a highly infested field resulted in an insignificant increase in faba bean yield compared to the unchecked one, due to damage being done before the parasite emerges. Late sowing of faba beans is a technique used by farmers to decrease infestation by *O. crenata*. In Egypt, faba bean planting is delayed 3 weeks to reduce *Orobanche* infestation (Al-

Menoufi, 1994). Unless early maturing varieties are available, such a delay in sowing results in a decrease in the crop yield. Still, Kharrat and Halila (1994) reported that delaying sowing by 35–40 days in O. foetida infested fields, halved the number of emerged Orobanche shoots and despite the shorter season increased yield by 20% due to reduced crop damage. Avoidance or reduction of growing Orobanche-susceptible crops through crop rotation is also an effective means to control the parasite. Long rotations that might help reducing infestations are unacceptable, as food legume production, which is strategically important in North Africa, will be reduced. For example, the loss of faba bean for many years would not be acceptable, as it is the most important source of human dietary protein in the region. The use of trap/catch crops has also been very limited by farmers, probably for economical reasons. Trap crops only partially reduce the *Orobanche* seedbanks, as many seeds do not germinate and remain viable in the soil (Garcia-Torres et al., 1994).

The effectiveness of soil fumigants such as methyl bromide (which is being phased out of use globally, due to environmental concerns) for the control of soil-borne pests and weed seeds has been known for a long time. One of the main uses of methyl bromide has been in horticulture for soil fumigation of seedbeds of crops such as tomatoes, peppers, eggplants, and melons. In general, its use has been restricted to high-value cash crops because of the costs involved. Despite the effectiveness of methyl bromide in controlling *Orobanche* spp., its use in the low input cropping systems is beyond farmers' means and in most cases is unaffordable as legumes are not considered as high value crops

<sup>&</sup>lt;sup>a</sup> Includes finger millets in the lake zone of east central Africa.

<sup>&</sup>lt;sup>b</sup>Includes both sorghum and pearl millet combined in West African countries only.

<sup>&</sup>lt;sup>a</sup>Loss includes sorghum, millets, and maize.

and the marketing system in Maghreb countries does not secure a high enough price. Al-Menoufi (1994) reported that soil solarization in irrigated soils in Egypt effectively controlled *Orobanche* in faba bean fields and increased yields four-fold. This method is also relatively expensive and faces the limitations stressed above.

The use of herbicides to control Orobanche has not been widely accepted by farmers. Glyphosate and the imidazolinone herbicides (imazethapyr, imazapyr and imazaguin) have been tested to control Orobanche in faba bean and other crops (Kharrat and Halila, 1994; Sauerborn et al., 1989; Zemrag, 1994). In Egypt, very low rates of glyphosate have been extensively assayed in faba beans and it was released for commercial use in this crop (Saber et al., 1994). Herbicide effectiveness would require repeated applications during the initial stages of the development of the parasite before emergence above the soil surface. Indeed, repeated vs. a single application of glyphosate provided better control of the parasite in faba bean. In Morocco, glyphosate application two times at 15 day-intervals is the most widely used control method in faba bean (Zemrag, 1994). In addition to repeated applications, the very limited margin of selectivity is another constraint to herbicide use by farmers (Jacobsohn and Levy, 1986). Glyphosate can be used at low rates in some legume and umbelliferous crops but not in many others such as tomatoes and peas (Jacobsohn and Kelman, 1980). It is doubted that a manufacturer of glyphosate will request adding these crops to the label due to possibilities of overdose and liability, as well as the small market potential. Additionally, the inadequate farmer knowledge of Orobanche biology coupled with the needs for timing of application, as well as climatic factors (temperature and rainfall) have limited glyphosate use by farmers (Saber et al., 1994; Yazough and Klein, 1999). When applied prior to emergence, some imidazolinone herbicides control Orobanche in faba bean, however their efficiency is inconsistent and affected by environmental conditions (Garcia-Torres et al., 1994; Kharrat et al., 2002).

Host resistance would be the most economic means to fight this parasitic weed. Considerable efforts have been expended by plant breeders in many countries to develop varieties resistant to *Orobanche*. Egyptian plant breeders developed and released Giza 402 faba bean, which shows some tolerance to O. crenata (Nassib et al., 1982), but is low yielding. Continued selection has led to a number of new lines with some Orobanche resistance (Cubero and Hernandez, 1991; Kharrat and Halila, 1999; Saber et al., 1999). However, resistance varies within each line and even for individual plants. The degree of resistance is inconsistent, and is strongly affected by the environment, and can be rapidly lost. Resistant/tolerant varieties with better quality characters such as large seeds, high yield, and good taste are not yet available. Unless such characters are present,

resistant varieties will not be widely accepted by farmers in the region.

3.2.3.2. Striga. Conventional techniques used to control Striga were described more than a decade ago under three so-called principles: reducing number of Striga seeds in the soil bank; preventing production of new seeds; and preventing spread from infested to noninfested soils (Berner et al., 1995; Hess and Ejeta, 1992; Obilana, 1984; Obilana and Ramaiah, 1992). Progress has been limited when using any of these approaches alone. However, using a combination of these three techniques in SSA, which include nitrogen fertilizer (Berner et al., 1995), hand pulling (Ramaiah, 1985), intercropping and crop rotations (Oswald and Ransom, 2001; Oswald et al., 2002), host plant tolerance using tolerant varieties (Haussmann et al., 2000a,b; Kling et al., 2000; Obilana, 1987; Rattunde et al., 2000; Wilson et al., 2000) and clean seed, achieved acceptable levels of control of Striga if practiced long enough. A better integrated approach for management and reduction of the Striga scourge was proposed for implementation, and includes components of the Obilana/Ramaiah principles, coupled with application of marker technology, and QTL (Quantitative Trait Loci) analyses for MAS (Marker-Assisted Selection) and research into Striga variability, the physiological basis of resistance in host plants and the use of biodiversity in wide crosses. These molecular breeding methods should be integrated with herbicide biotechnology (see Section 3.2.4.1), with biocontrol (Marley et al., 1999), technology exchange with farmer participation, and non-conventional approaches to reduce Striga vigor and infestation by genetic engineering.

More recently, a novel pest management approach based on a 'push-pull' or stimulo-deterrent diversionary strategy was developed using trap- and repellent plants. As part of this kind of approach, Khan et al. (2002) demonstrated the benefits of plant diversity in order to reduce Striga infestations in maize-based farming systems. The system uses fodder plants intercropped in maize to control pest and weed infestation. They reported Striga suppression by Desmodium (a perennial fodder plant used in habitat management), which fits in well with some small-to-medium-scale farmers' practice of mixed agriculture in certain areas. It may not be widely adopted, as most subsistence farmers need most of their land for staple food crop production, and the few that have animals, do not cultivate fodder crops specifically for them. It also requires fencing to protect the legume from grazing. Perhaps once yields are much higher due to *Striga* control in the food crops, farmers can augment this with such fodder crops as part of an integrated management package.

None of these systems has been widely adopted by farmers, suggesting that they are not fully adequate or cost-effective, or that they do not easily fit with farming practices. Thus, for sustained *Striga* control and management, it is imperative to foster new integrated approaches including biotechnological solutions, with concerted resource mobilization, wider strategic partnerships, novel multidisciplinary linkages and participatory approaches with farmers.

### 3.2.4. Potential biotechnological solutions and status

Many different biotechnological approaches have been taken to deal with parasitic weeds, but only two have been subjected to more than a few field trials, as will be discussed below.

3.2.4.1. Herbicide resistant crops. The possibility that the parasites might be controlled by systemic herbicides applied to herbicide resistant crops was suggested over a decade ago (Gressel, 1992), but it took a few years to obtain transgenic crops to validate the concept with Orobanche and model crops such as tobacco and oilseed rape (Joel et al., 1995). Since then, potatoes (Surov et al., 1998) and carrots (Aviv et al., 2002) were specifically transformed with herbicide resistance genes, and the concept was demonstrated to work with transgenic glyphosate-resistant tomatoes (E. Kotoula-Syka, pers. comm.). The concept should work equally well with glyphosate-resistant maize, but such material has not been made available to researchers who wished to test it. The effect of the transgenes encoding a modified acetolactate synthase conferring resistance to a wide range of herbicides affecting that target can be achieved by standard mutagenesis. The gene is highly mutable and maize resistant to this group of herbicides was first obtained by tissue culture selection for mutants and regeneration (Newhouse et al., 1991), and later, far more simply, by pollen mutagenesis (Greaves et al., 1993). Such maize from the USA was crossed and backcrossed by CIMMYT into African open pollinated varieties and inbreds for hybrid production (Kanampiu et al., 2003b). Methods were developed to apply small amounts of herbicide to seed (>10-fold less than would be sprayed on a field), precluding the need for expenditures on equipment (Abayo et al., 1998; Kanampiu et al., 2001). The seed treatment is "appropriate" for African farming regimes, as intercrops of legumes are not affected by the treatment (Kanampiu et al., 2002). Large scale experiment station and farmers' field trials have been ongoing in four east African countries, with yields nearly tripled on average (Kanampiu et al., 2003b). A major advantage is that the herbicide provides season long control in short season, double cropped maize in western Kenya, at even the highest Striga infestation levels; yields are normal with treated maize, where untreated maize has a total loss (Kanampiu et al., 2003a, b). Even when there are some late season attachments by the parasites, they did not set seed

before harvest, so there is no replenishment of the seed bank. CIMMYT has released material to local seed companies for bulking up and varietal registration (Kanampiu et al., 2003a). Similarly, sunflowers with the same type of mutation are being released in eastern Europe for *Orobanche* and general weed control, but whole-field spraying will be used to control other weeds as well.

Because of the high natural frequency of resistance, modeling suggested that there is a large risk that resistance could rapidly evolve to this group of herbicides (Gressel et al., 1996) and monitoring will be instituted. There is the possibility that the modeling overstated the risk. The models were predicated on the heterozygote mutation frequency for this trait, because it is typically dominantly inherited at the doses used in other crops. Despite the use of a low dose per hectare, there is the possibility that resistance must be homozygous to withstand the very high dose in the immediate vicinity of the treated maize seed. If that were the case, resistance would be much longer in coming because of the low frequency of recessive homozygous mutations.

3.2.4.2. Biological control. Both insects and fungi have been isolated that attack these parasitic weeds (see review by Amsellem et al., 2001b). The insects attack mainly the seedpods, eating most, but never all of the seeds. Thus, replenishment of the seed bank is sufficient to sustain the weed population (Smith et al., 1993) while having little yield promotion, and thus insects will not be further discussed.

Various fungi have been tested both for pathogenicity on *Striga* (Ciotola et al., 1995, 1996, 2000; Marley et al., 1999; Savard et al., 1997) and *Orobanche*, (Al-Menoufy, 1986; Amsellem et al., 1999, 2001a; Bedi and Donchev, 1991; Linke et al., 1992; Thomas et al., 1999), but none are yet in wide scale field testing. Regulatory authorization may be a problem for even indigenous strains, as the best are formae speciales of *Fusarium oxysporum* that are specific to the parasite and not the host. DNA fingerprinting (Amsellem et al., 2001a) suggests that these strains diverged from crop-pathogenic strains of this species over 100,000 years ago (L. Hornok, pers. comm.), yet regulators are fearful of all *F. oxysporum* formae speciales.

The strains of *F. oxysporum* and *F. arthrosporioides* that attack *Orobanche* (Amsellem et al., 2001b) have not been successful in providing near the level of control desired by farmers when tested in the field. Transgenes encoding auxin production were introduced into an *Orobanche*-attacking fungal species, significantly doubling virulence (Cohen et al., 2002), which is still far less than what the farmer needs. Far stronger toxic genes are needed to enhance virulence, and the NEP1 gene, used to enhance a different mycoherbicide (Amsellem et al., 2002) also was active in enhancing the virulence of a

F. arthrosporioides that is specific to Orobanche spp. (Amsellem and Gressel, recent results). The biosafety aspects of using transgenically hypervirulent biocontrol agents are specifically addressed in Gressel (2001, 2002, 2003).

While intercropping of parasite-susceptible and resistant crop species is not considered to be "biocontrol" an interesting allelochemical compound has been described from the research using *Desmodium* as an intercrop (Khan et al., 2002). This could also be the case for sesame intercropped with sorghum and pearl millet to control *Striga* in Eritrea and some other western African countries (ICRISAT, 2002). If parasite-susceptible crops could be engineered to produce this compound (when the genes responsible are found, and if not too many) resistance might be possible, without "intermediaries", i.e. herbicides, biocontrol agents or intercrops.

3.2.4.3. Engineering crops for direct resistance to parasitic weeds. There are some crops "trap crops" that are attacked by parasitic weeds yet are unaffected. They must have the right genes for conferring resistance to other crops. To the best of our knowledge there is no ongoing research to find these genes, a rather complicated approach possibly being somewhat simplified with new "DNA chip" technologies for finding useful genes.

A simpler approach is to have the crop root emit a toxin (Duke, 2003). As there is a metabolic cost to constitutively producing toxins, as well as a possibility of autotoxicity, the approach has been taken to put such toxins under promoters that are activated by parasite attack. Such a promoter has been isolated and demonstrated to strongly activate a reporter gene (Westwood, 2000; Westwood et al., 1998). Preliminary experiments have been reported that this also works with an antibiotic that has anti-plant activity as well (Aly and Plakhine, 2001).

There has been a theoretical proposal to engineer *S. hermonthica* with a multiple copy transposon containing a lethal gene under the control of an inducible promoter (Gressel and Levy, 2000). This is based on a concept proposed and partially tested for the control of insect populations (Grigliatti et al., 2001). The transformed *Striga* would be released in the field, and all crossed

progeny would bear the gene construct (instead of half, as with Mendelian backcross inheritance). After the construct spreads through the population, the gene could be turned on, either by a systemic chemical applied to the crop, or a new crop variety secreting the chemical inducer. *S. hermonthica* is the only one of the major parasitic weeds that is an obligate outcrosser, and thus the only one where this concept might work.

# 3.3. Bemisialtomato yellow leaf curl virus (TYLCV) constraint in northern Africa

Plant viruses and their insect vectors represent a dramatic example of how two types of pests "team up" to cause major problems for African vegetable production. The vast movement of people and agricultural products between Africa and distant geographical regions has created unprecedented opportunities for introducing plant viruses and the insects that carry viruses to new areas. Outbreaks of new viruses were abetted by crop susceptibility, the presence of weeds that acted as alternative hosts and inadequate control practices. The conditions were often ideal for the emergence of altered plant viruses and new virus/vector relationships. This resulted in the appearance of insecttransmitted plant viruses in crops and regions where they have not been seen before. Because plant viruses and their insect vectors are intimately linked, the status of both must be considered in formulating strategies to prevent or slow their introduction, as well as to manage any invasions.

The recent invasion of North Africa by TYLCV, which was introduced to Tunisia and Libya in 1994, and spread to Morocco in 1998 (Hanafi, 2000, 2001) and to Algeria in 2000, have resulted in immense economic losses to the farmers and the economy (Table 8). The virus is exclusively transmitted by the whitefly *Bemisia tabaci*, which was already present in North Africa. *Bemisia* is an ideal agent for spreading the virus because of its high rate of reproduction, ability to disperse, and its obligate use of particular plants.

North African farmers had already been faced with a plethora of other insect-transmitted viruses that can limit crop production, and for which there are few effective management options. This new virus

Table 8
Tomato yellow leaf curl damage to spring field tomatoes

Country	Area planted ('000 ha)	Yield (tons/ha)	Production ('000 tons)	Area affected (%)	Est. yield loss (%)	Value lost (million \$)
Morocco	14	30	420	50-100	65–100	245–404
Tunisia	18.5	25	462	25-90	30-100	179-450
Libya	18.7	22	411	60-100	50-80	79–151
Egypt						343–472

Compiled by A. Hanafi.

constituted a challenge to north African greenhouse production systems that for a decade had rapidly evolved towards greater IPM with less than 10 insecticide applications during a tomato crop cycle. The appearance of TYLCV has boosted the use of insecticides up to 68 per tomato crop cycle in Morocco (Hanafi and Papasalomontos, 1999).

In contrast to many diseases caused by whitefly transmitted geminiviruses, TYLCV is not a new problem. This disease was first described in Israel around 1940, and was associated with outbreaks of the sweet-potato whitefly. TYLCV was subsequently described in parts of the Middle East, Africa, Southern Europe, India and Asia. The causal agent of TYLC was first identified as a geminivirus in 1988. The geminivirus (Geminiviridae) family is characterized by twinned (in Latin, gemini is twin) icosahedral virus particles. Geminiviruses can be carried by either leafhoppers or whiteflies, and whitefly transmitted geminiviruses are one of the major emerging groups of plant viruses world wide, and it is now known that a number of distinct geminiviruses cause tomato yellow leaf curl-like symptoms in different parts of the world.

The infected leaves turn yellow except for the veins, show strong upward curling of the outer leaf margins and are small and crumpled, and thus the name. Plants infected at a young age are severely stunted and new shoots grow straight up, resulting in small, compact plants with bushy tops that are commonly referred to as "bonsai" plants. In general, the younger the plant at time of infection, the more severe the stunting. Tomatoes infected at an early stage of growth with TYLCV fail to produce fruit even though the infected tomato plants produce abundant flowers. The virus causes flowers to abscise long before fruit setting. The relative age of plant infection can be easily determined because only the new growth shows symptoms. Field yield losses can reach 100%.

There are at least two possible ways new whitefly transmitted geminiviruses could have entered/appeared in North Africa: (1) introduction via infected plant material or viruliferous whiteflies on plant material; and (2) possible introduction via passive flights of viruliferous whiteflies from Spain, 14km from Morocco. Whiteflies can be carried for longer distances by winds. B. tabaci had previously been reported in the Mediterranean region and had probably been also inadvertently introduced in the past into North Africa, through infested plantlets (such as strawberries or tomato transplants). At least two biotypes of the vector have been identified so far in North Africa, biotype Q is endemic and biotype B is invasive and is behind much of the spread of TYLCV into new geographical areas (Hanafi et al., 2002).

TYLCV is established throughout northern Africa (Table 8), causing enough economic damage that many

small farmers abandoned open field tomatoes. TYLCV would probably be difficult if not impossible to eradicate from northern Africa due to the diversity of crops grown and the wide host range of the whitefly. These factors render it difficult to implement solutions that have helped elsewhere, such as host-free periods.

### 3.3.1. Conventional technologies that are inadequate or are very expensive

In response to the increased to the need for pesticide applications, growers have been adopting IPM tactics focused primarily on prevention of disease transmission. A number of tactics have been implemented with some degree of success, including tolerant cultivars, roguing diseased plants, and alternating planting dates. For managing the vector, *B. tabaci*, farmers have adopted improved pest monitoring and control guidelines, mass trapping, exclusion nets, and pesticide rotation to preserve effective pesticides. However, the two tactics most relied on by farmers are chemical controls and exclusion nets to reduce disease transmission by *B. tabaci* (Hanafi, 1999, 2003).

3.3.1.1. Insect nets for the exclusion of the vector. Over 90% of greenhouses now use insect nets of various mesh gauge sizes to exclude B. tabaci from greenhouses (Hanafi and El Fadl, 2002; Hanafi et al., 2003c,d). Considering the time and expense devoted to exclusion nets, evaluations were begun to determine the costs and benefits of using insect nets within the overall IPM program. Factors such as the effect of mesh size on disease incidence and B. tabaci, impact on biological control, disease management and crop yields were included. The first objective of the program compared the efficacy of two mesh sizes most commonly used by farmers. The  $10 \times 14$  threads/cm screens are neither efficient in excluding Bemesia nor in preventing TYLCV, generating a heavy reliance on insecticides. The superiority of the  $10 \times 20$  threads/cm screen was clearly demonstrated in terms of whiteflies captured and the final TYLCV incidence, and was best in greenhouses constructed to avoid the smallest possible holes, with insect screens on all ventilation openings (roof and laterals) long before planting. The problem with such fine mesh screens is that in the dusty conditions that prevail in northern Africa, airflow resistance becomes a problem, requiring a costly 30% greater screening area to maintain adequate natural ventilation (Hanafi et al., 2003a, b).

3.3.1.2. Application of chemicals (especially those not harmful to beneficial insects) and resistance. Until recently Moroccan tomato farmers knew very little about the whitefly problems. Populations of whiteflies in greenhouse crops were mainly those of *Trialeurodes vaporariorum* representing over 80% of total whitefly

populations in greenhouse tomatoes. Even then, insecticides were never required to control this pest, which was under natural biological control by several natural enemies, mainly *Cyrtopeltis temuis* in greenhouses that were semi-open (no insect netting was used in the greenhouses).

The introduction of TYLCV to Morocco coupled with the presence of the vector B. tabaci caused a panic reaction among farmers. The approach to whitefly control then became the use of organophosphate, carbamate, synthetic pyrethroid, or chlorinated hydrocarbon insecticides in the greenhouse. Stomach acting insecticides that remain on the leaf surface were useless against whiteflies, as their proboscis penetrates through droplets of stomach poison into the plant tissue below. Contact insecticides must come in contact with the insect to be effective, so thorough coverage is necessary. Currently, the pesticides most commonly used for whitefly control are endosulfan, methomyl, pyriproxyfen, thiamethoxam, and novaluron. Some growers mix two chemicals with two modes of action in the same application. The reasoning is sound, because if the population includes whiteflies resistant to one of the chemicals, the other will kill them. This heavy reliance on insecticidal control using all available compounds at a high frequency has resulted in the evolution of resistance by Bemisia to some compounds that were initially very effective. During the last 2 years we have detected various level of resistance to imidacloprid, cypermethrin, deltamethrin, thiamethoxam and methomyl (Hanafi et al., 2003a; Bouharroud et al., 2003).

3.3.1.3. Integrated pest management (IPM). While most of the individual components of an IPM program are not new, the increasing popularity of combining them into an integrated production and protection (IPP) systems is new and are being adopted for very good reasons. Regulations regarding pesticide residues in exported agricultural products are likely to increase in number and severity, especially in Morocco, Tunisia, and Egypt. Worker protection regulations, economics, the registration process, public opinion, and most of all advances in technology have brought these systems approaches to the forefront. The integration of improved sanitation, weekly scouting and monitoring with yellow sticky traps, better record keeping, the use of screens or barriers to prevent insects from entering greenhouses (described above), inspection, isolation and treatment of incoming planting material to prevent infestations, and insecticides all continue to play an important part of IPM programs. The use of parasitic and predator insects and beneficial fungi (see below) may play a major future role in IPM, and breeding and use of TYLCV tolerant cultivars are needed.

Resistance to TYLCV should really be the first line of defence for open field tomatoes, which represents the bulk of the production in North Africa. Unfortunately, many of the commercially available tomato cultivars that are tolerant/resistant to TYLCV lack also the agronomic and quality requirements and seed is 80–150% more expensive than conventional varieties. All commercially available tomato cultivars are the result of conventional breeding, which is expensive and time consuming, and there is a good question as to whether the tomato genome has sufficient genetic diversity to deal with the pest and the virus, which evolved in a different part of the world from tomatoes. Biotechnology, by bringing in resistance genes from other species could probably offer a breakthrough by developing adequate resistant/tolerant cultivars and which also have the required quality for farmers and consumers.

3.3.2. Potential biotechnological solutions and status 3.3.2.1. Biocontrol of the vector. Predators and parasitoids have been registered for use in biological control in Morocco and Tunisia since 1992. Koppert Biological Systems is continuing to develop IPM packages adapted to local conditions in Morocco and Tunisia. Biobest Maroc produces natural enemies in Morocco. Encarsia formosa, a small parasitic wasp is the chief natural enemy of the greenhouse whitefly (T. vaporariorum). Several companies supply *Encarsia* for control of *T*. vaporariorum in the pupal stage, the stage most tolerant to environmental extremes and therefore most able to survive the rigors of shipping. Encarsia has limited efficacy against the sweet potato whitefly (B. tabaci), the vector of TYLCV. However, Eretmocerus sp. gives excellent control of this pest. This parasitic wasp is being used successfully in Morocco and Spain for the control of B. tabaci.

These predators and parasites are very sensitive to many chemicals farmers apply to control other pests and diseases, and the farmers are provided with information on the side effects of traditional pesticides on natural enemies. Sprays of some chemicals can be toxic even when applied months before parasitoid release.

It must be noted that biocontrol has been used only in greenhouse situations and the organisms used are not contemplated for field use.

3.3.2.2. Genetic engineering to control the insect vector. Possible alternative approaches to controlling tomato leaf curl virus include technologies to control the whitefly vector and technologies to directly combat the virus itself. To achieve the former, insecticidal proteins with homopteran activity would be needed, together with an expression system that could target the protein to the appropriate tissues in tomato leaves where whiteflies feed. Suitable proteins could include those derived from the bacterium Bacillus thuringiensis, particularly the relatively broad spectrum Vegetative Insecticidal Proteins (Vips) (Estruch et al., 1996;

Schnepf et al., 1998), or plant-derived proteins such as lectins or proteinase inhibitors. Thus far, neither crystalline Bt proteins nor Vips have been identified to have homopteran activity (for example, see Bernal et al., 2002). However, both lectins and proteinase inhibitors have been used successfully in transgenic rice cultivars to control homopterans like the brown and green planthoppers (Foissac et al., 2000; Lee et al., 1999).

One potential downside of this approach is that insecticidal proteins may control the whitefly vector without preventing some amount of disease transmission, because the insects must feed on tomato plants to be exposed to a plant-expressed insecticidal protein. If that amount of transmission is sufficient to cause economically important levels of damage, then this approach to controlling tomato leaf curl virus may not be viable.

3.3.2.3. Genetic engineering to control the virus. In contrast, an approach that directly combats the virus could provide complete protection, as has already been demonstrated commercially in potato with several different sorts of viruses. For example, transgenic cultivars with engineered control of potato leaf roll virus (NewLeaf Plus) and potato virus Y (NewLeaf Y) have been commercialized in North America (Krohn et al., 1998; Thomas et al., 2000). Suitable technologies could involve coat protein recognition, as used with potato viruses X and Y, or encoding viral replicase proteins, as has been used with potato leaf curl virus.

As viruses can evolve mechanisms to overcome these types of resistance, and insect vectors can evolve resistance to insecticidal proteins, just as they have to insecticides, the best approach would probably be to stack one or more anti-vector proteins with one or more virus resistance mechanisms, to provide a longer lasting resistance.

# 3.4. Maize stem borers (Chilo partellus and Busseola fusca) in sub-Sahara Africa

Pre-harvest crop losses due to stem borers account for over 20–40% in maize and sorghum production in Africa (DeGroote, 2002) (Table 9). The tropical environment provides a highly favorable climate for

Table 9
Estimated production losses due to stem borer in Africa

Total affected	16.48 million ha
Estimated yield loss Estimate of total annual loss in production Total annual production Potential annual loss	20% 3.9 million tons 19.5 million tons \$ 390 million <sup>a</sup>

Compiled by J. Ochanda.

arthropods, especially Lepidopteran pests. Yield losses due to insect damage range from 25% to 40% reaching 80% in serious infestations and 40–80% in stored products. The main pests causing serious yield loss in Africa are stem-borers, *Chilo partellus, Busseola fusca, Sesamia calamisties* and *Chilo orichal-cociliellus* (Youdeowi, 1989; Seshu Reddy, 1983; Sithole, 1989). Most of the damage is due to *Chilo partellus* and *Busseola fusca* (Seshu Reddy, 1983).

Stem borers are one of the most important pests of maize and sorghum. Estimates of losses under natural infestation and the incidence of infestation are used to estimate yield loses for each of the Sub-Saharan African countries (Kfir, 1997). For example in Kenya, the maize yield loss was estimated to be 13%, amounting to 0.4 million tonnes of maize, with an estimated value of US\$ 76 million.

Stem borers seriously limit potentially attainable maize yields by infesting the crop through out it growth, from seedling stage to maturity. Seventeen species which belong to families Pyralidae and Noctuida have been found to attack maize in various parts of Africa (Warui and Kuria, 1983; Sithole, 1990). The most important species of stem borers are the spotted stem borer Chilo partellus and Busseola fusca found in cooler and higher altitudes (Ajala and Saxena, 1994). The less important species is Sesamia calamistis found at elevations up to 600 m. On farm test plots of maize, the average yield losses due to stem borers range between 17-23% and 10-17% (Sithole, 1989; Brownbridge, 1991). The yield loss caused by stem borers to maize varies widely in different regions and range from 20% to 40% according to the pest population density and phenological stage of the crop at infestation (Seshu Reddy, 1989).

### 3.4.1. Conventional technologies that have failed

African farmers have developed technologies for inexpensively using small amounts of insecticide to cope with the problem of stem-borers; technologies that hardly approach commonly accepted safety standards. They either bare-handed put a pinch of a granulated formulation of an organophosphate insecticide into the whorl of leaves of the maize or sorghum plants, or similarly apply a few drops of a liquid formulation with a medicine dropper. In some cases, baited traps with female sex pheromone have been used (Lux et al., 1994). However, for reasons that include unsuitability, unavailability and high cost, many resource poor farmers do not use any control methods, and thus lose a significant amount of their potential yields. The insecticidal procedures described above are not as simple, inexpensive or as safe as the biotechnologies described below.

Scientists at ICIPE have recently developed a method called the "push-pull" strategy for minimizing the stem borer infestation in crop fields. The approach makes use

<sup>&</sup>lt;sup>a</sup> Value calculated at \$100/ton.

of the underlying chemical plant defense mechanisms. Two legume species, *Desmodium uncinatum* and *D. intirtum* have shown some positive results. However, the application is complicated and might require expertise the rural farmers lack. In practice, the maize field is planted with a permanent stand of the perennial *Desmodium* between the rows of maize to repel the borers, and Napier grass is planted on the field edges to trap borers from the crop (Khan et al., 2001). The pushpull procedure is limited to certain geographies, is complicated and expensive to establish, yet is appropriate where farmers need the forage crop for animals, and can cope with the intricacies.

# 3.4.2. Potential biotechnological solutions and status: Bt genes engineered in crops

Biotechnology has some very effective solutions for stem borers in maize, as has already been demonstrated on a global basis. The important maize stem borers of sub-Saharan African consist of Lepidoptera from two families: Pyralidae and Noctuidae. Substantial industrial and academic research in Africa, Asia and the Americas has demonstrated the efficacy of a variety of crystalline proteins from *B. thuringiensis* (Cry proteins) against these insect groups (e.g. (Mugo et al., 2001a-c; Schnepf et al., 1998; Van Rensburg, 1999). In general, Cry proteins in Classes 1 and 2 display insecticidal activity against these and other families of lepidopteran pests. For this reason, transgenic maize products expressing either a Cry1Ab or a Cry1F protein have been globally commercialized, including in the United States, Canada, Argentina, South Africa, the Philippines and Spain. These products are capable of very effectively controlling pyralid moths such as those in the genus Chilo, and provide partial to very good control of noctuid moths in the genera Sesamia, Busseola, and Spodoptera (e.g. Gonzalez-Nunez et al., 2000; Van Rensburg, 1999; Williams et al., 1997). For example, trials being conducted by CIMMYT and KARI in Kenya under the IRMA (Insect Resistant Maize for Africa) project have demonstrated considerable success in the control of Chilo partellus, Eldana saccharina and Sesamia calamistis (Mugo et al., 2000, 2001a-c). Other candidate Cry proteins that have been extensively tested by industry and/or public laboratories are the Cry1B's and the Cry2A's. In addition, several Vip proteins from B. thuringiensis have been shown to have broad lepidopteran activity (Estruch et al., 1996; Schnepf et al., 1998). Thus, there are a variety of insecticidal proteins in the public and private domain that could be genetically engineered into maize (and already have been in other regions) to provide effective protection from stem borers. To ensure high levels of control of all the lepidopteran pest species, multiple proteins could be simultaneously introduced, as has been done with transgenic cotton in the United States and Australia, where the cotton expresses both Cry1Ac and Cry2Ab (Greenplate et al., 2003). By employing a constitutive promoter, all relevant maize tissues could be protected in a way that is not possible when insecticides are applied by spraying; in particular, stem and cob tissues that are favored by the stem-borers are not accessible to spraying but can be completely protected with a plant-expressed insecticidal protein. Furthermore, Cry proteins are highly specific in their insecticidal properties, thereby limiting any non-target effects and removing the hazards to human health represented by some conventional insecticides (Betz et al., 2000).

### 3.5. Grain weevils in sub-Saharan Africa

The other set of insect pests causing massive crop losses in SSA is the storage grain weevils, especially the larger grain borer, which causes a loss of 40-60% in storage (Table 10). The grain weevils (Curculionidae) are well known as major primary pests of stored cereal grains and have spread throughout most of the African continent. They are able to establish themselves on whole, undamaged grains of maize, sorghum, rice and wheat as long as the grain is not exceptionally dry. Sitophilus zeamais is the dominant species on maize while Sitophilus oryzae is dominant on wheat. The bostrichid beetle Prostephanus truncates (the larger grain borer) is a highly destructive primary pest of maize, especially maize stored on the cob. This insect is now established in several eastern and western African countries following recent accidental introductions from

Constraints to food production in Africa due to grain weevils

	-			
Country	Area planted ('000 ha)	Yield (kg/ha)	Estimated yield loss (%)	Yield loss ('000 ton)
(A). Large grai	n borer in m	aize		
Botswana	83	112	19–27	1.8 - 2.5
Cameroon	350	2429	31	263
Congo	1463	799	18-28	210-327
Ghana	713	1315	20.0	188
Kenya	1500	1800	23-41	621-1107
Malawi	1446	1099	14–18	222-286
Mozambique	275	896	23-39	57–96
Sierra Leone	10	928	40	3.6
Tanzania	1457	1795	34	889
Uganda	652	1801	50	2114
Burundi	155	1087	29–47	49–79
(B) Pod borer	Maruca vitra	ta in cowpe	as (countries w	here data
available)			•••	
Malawi	79	683	30–88	16–47
Senegal	146	323	12–70	6–33
Niger	3000	117	23–60	80–210
Tanzania	147	320	18–83	8–39
Kenya	150	484	32–71	24–51

Compiled by J. Ochanda.

its previously more limited indigenous range in meso-America (Dick, 1988; Golob, 1988; McFarlane, 1988).

### 3.5.1. Conventional technologies that have failed

The evolution of insecticide resistance is a well-known pest management problem common to many species of storage insects, and to a wide range of insecticides (Champ and Dyte, 1976), and stems from careless use, which is common among Africa's resource poor farmers. As a consequence, the only available methods of control of grain weevils involve the use of ineffective and expensive chemicals to which the target species have evolved high resistance. There are other comparable approaches employing the use of neem extracts (Saxena, 1994) but these methods are not affordable by small holders.

Cultural control strategies have been employed to control grain storage pests. Grain moisture content considerably affects pest status but is not a factor that can be cost-effectively manipulated by artificial drying in most humid tropical situations to achieve sufficient control of insect pests, especially with farmer-stored grain for household use. Insect development and growth rates are dramatically enhanced by warm tropical African temperatures and the pests appear to develop much faster under storage conditions. The only traditional concept of sealed (hermetic) storage as a means of controlling insect infestation depends on reduced oxygen availability (Hyde et al., 1973). Concepts of reducing resistance by genetic control also have progressed to some extent (Wool et al., 1992) and are not implausible. However, their practicality and cost effectiveness in stored-grains pest control remain undemonstrated.

### 3.5.2. Potential biotechnological solutions and status

The use of Bt genes and other biotechnological approaches therefore appear to be most practical possibilities for effective control of these serious pests threatening food security stability in Africa.

As with stem-borers in maize, genetic engineering of insecticidal proteins into the crops of interest could effectively control grain weevils. Unlike the case of stem borers, suitable Cry proteins have not yet been identified for grain weevils. Cry3 proteins are known to have coleopteran activity, as are the binary proteins in classes Cry34 and Cry35 (Ellis et al., 2002; Schnepf et al., 1998). In addition, some Vip proteins have broad coleopteran activity (Carrozzi et al., 1993; Schnepf et al., 1998). Thus, screening of a number of proteins from B. thuringiensis for weevil activity would be a logical first step. In addition, various proteinase inhibitors have been shown to be capable of partially controlling certain weevil species (e.g. Girard et al., 1998), so these could also be a source of suitable insecticidal proteins. If a suitable protein can be identified, then currently used

expression systems have already been shown to produce levels of expression in the grain capable of protecting the grain against storage pests, e.g. lepipoteran pests such as the grain moth are largely controlled by low levels of Cry1Ab expression in grain, see Sedlacek et al. (2001). As with stem borers, the use of highly specific, plant-expressed insecticidal proteins would remove the human health risks posed by fumigants.

### 3.6. Mycotoxins (aflatoxins and fumonisins) in sub-Saharan Africa

Mycotoxins are secondary metabolites of fungi that are toxic to animals and man. Mycotoxins acutely as well as chronically affect both animals and humans. Acute outbreaks of mycotoxicoses are the tip of the iceberg (referred to in Africa as the ears of the hippopotamus). The chronic insidious effects of mycotoxins such as growth stunting, immune suppression and cancer are much more important although they may not be so evident (see reviews by Marasas, 2001a, b; Pitt, 2000; Staib et al., 2003; Turner et al., 2002). The fungi that produce the mycotoxins infest the crops systemically, often with stem borers and grain weevils as the vectors, or causing the lesion through which the fungi enter the crop plant or seed.

Mycotoxin constraints in staple foodstuffs in SSA are caused mainly by two carcinogenic mycotoxins, aflatoxin and fumonisin. Aflatoxin was first identified in a shipment of peanut meal contaminated with Aspergillus flavus from Brazil (Lancaster et al., 1961). Subsequently, aflatoxin was shown to cause outbreaks of acute hepatitis in animals and humans, to cause liver cancer in animals, and to be associated with liver cancer in humans, particularly in SSA and Southeast Asia. Scientists have observed a strong association between impaired childhood growth (Gong et al., 2002), and the seasonal appearance of the disease Kwashiorkor in children, with the seasonal presence of aflatoxins in the diet (Hendrickse, 1999). Susceptibility to disease in children is also enhanced by aflatoxins due to a suppression of immune function (Turner et al., 2003). A voluminous literature on aflatoxin has appeared during the past four decades involving thousands of publications.

Fumonisins were first isolated in South Africa in 1988 from cultures of *Fusarium verticillioides* (=*F. monili-forme*) (Gelderblom et al., 1988) and their structures were elucidated (Bezuidenhout et al., 1988). There were widespread outbreaks of leukoencephalomalacia in horses and pulmonary edema syndrome in pigs fed corn screenings in 1989/1990 in the USA, apparently caused by fumonisin B<sub>1</sub> (Harrison et al., 1990; Kellerman et al., 1990) based on an observed association between naturally occurring levels reported in corn screenings with the field outbreaks of these diseases (Plattner et al.,

1990). Fumonisins were also found to occur naturally in home-grown maize in a high-incidence area of human oesophageal cancer in the Transkei region of South Africa (Sydenham et al., 1990) and to cause liver cancer in rats (Gelderblom et al., 1991). Fumonisin B<sub>1</sub> inhibits folic acid transport and the deficiency causes neural tube defects and birth defects in humans (Stevens and Tang, 1997). A provisional maximum tolerable daily intake of 2 μg fumonisins/kg body weight was determined for these human carcinogens (WHO, 2002). Such regulations, when enforced in Africa, are enforced for the urban population, and not the 80% of the population who are subsistence farmers. The problems are especially acute with farm-stored grains for household usage, which invariably contain much higher levels than material sold commercially. Such maize is not included in legislation concerning maximal tolerable levels of fumonisin B<sub>1</sub> and aflatoxin in commercial maize. A synergistic interaction between fumonisin and aflatoxin in eliciting cancer has been demonstrated (Gelderblom et al., 2002). The risk of FB<sub>1</sub> is determined by both maize intake and level of contamination. The greatest maize consumers in rural areas also consume the most highly contaminated home-grown maize (Gelderblom et al., 1996; Marasas, 1997).

Fumonisins and aflatoxins occur in maize and other grains worldwide, including throughout Africa (Table 11). They occur in processed products, from those purchased by normal households through to heroin, as they are not completely degraded by heat. These toxins even make their way through the food chain into beer (Scott, 1996) and aflatoxins (but not fumonisins) carry over into milk (Galvano et al., 1996), including the milk of nursing mothers (Zarba et al., 1992).

In addition to the direct health problems, especially of subsistence farmers eating high intakes of stored grains, and the loss of grain yield inflicted by the pathogens, there has also been a loss of \$670 million in exports to Europe, as estimated by the World Bank. This is due to new EU standards (Otsuki et al., 2001), which would reduce health risks by approximately 1.4 European deaths per billion population per year, in contrast to international standards. These data have been extrapolated globally by Wilson and Otsuki (2001). A more serious consequence than the monetary loss is the fact that the aflatoxin-contaminated foodstuffs rejected by Europe will be consumed in Africa, where the populations are at much higher risk for primary liver cancer because of nutritional deficiencies and hepatitis B virus infection.

Lubulwa and Davis (1995) estimated the social costs of the impacts of aflatoxins in corn and peanuts in Indonesia, Philippines and Thailand using economic models that calculate the cost of disability due to aflatoxin-related primary liver cancer in humans and suppression of growth of poultry and pigs. The total annual social cost in these three countries due to aflatoxin in maize was Australian \$319 million (Table 12). The estimate for aflatoxin in peanuts was Australian \$158 million. Unfortunately, there has been no attempt to make such estimates in Africa.

### 3.6.1. Conventional technologies that have failed

Fungicides have been widely used in the developed world to control the toxigenic fungal infections of grains, but are only affordable for export crops in Africa. The effectiveness of fungicide usage in many cases is limited (D'Mello et al., 1998) due to a lack of consistency as well as to the evolution of fungicide resistance.

Folic acid reduces the risks associated with mycotoxins and fortification of all grain products with 1.4 pg/kg folic acid is now compulsory in the USA, and soon will

Table 11		
Recent studies on post ha	rvest levels of mycotoxins i	n African grains

Locale	Commercial/ Farmer storage	Main findings	Ref.
Benin		Parasitic insects on grain weevils reduce infestation by 10%	Setamou et al. (1998)
Benin	Farmer	Heat/humidity/insects/correlated with aflatoxin	Hell et al. (2000a, b)
Botswana		Aflatoxins in 40% sorghum and peanut samples, fumonisins	Siame et al. (1998)
Burundi		High levels of fumonisins in maize and sorghum	Munimbazi and Bullerman (1996)
Kenya	Farmer	Fumonisin levels above $1 \mu g/g$ in 5% of samples	Kedera et al. (1999)
Nigeria	Markets	High aflatoxin in 40% cassava samples	Ibeh et al. (1991)
South Africa	Supermarkets	Fumonisins low in commercial maize	Sydenham et al. (1991), Schlechter et al.
(and USA)			(1998)
Egypt		Fuminosin producing Fusarium spp. in maize ears	Allah (1998)
		High incidence of fumonisins in maize	Sydenham et al. (1991)
Africa (and		High fumonisin contamination Italy, Portugal, Zambia, Benin	Doko et al. (1995)
Europe)		maize samples	

In many cases the sample sizes are small.

Mycotoxin levels worldwide have been reviewed by Shepard et al. (1996) and by Placinta et al. (1999).

Table 12 Estimated annual social costs of aflatoxins in maize in Indonesia, Philippines and Thailand (1991)

Sector	Impact factor Social parameter estimated		Cost (M Aus\$) <sup>a</sup>	
Commercial grain	Spoilage effects	Wastage/postharvest costs	71	
Household	Human health	Disability from liver cancer	64	
	Human health	Premature death from liver cancer	113	
Poultry-meat	Reduced feed efficiency/increased mortality	Increased cost vs. aflatoxin-free Feed	29	
Poultry—eggs	Reduced feed efficiency/increased mortality	Increased cost vs. aflatoxin-free feed	7	
Pig meat	Reduced feed efficiency/increased mortality	Increased cost vs. aflatoxin-free feed	36	
Total			319	

Condensed from Lubulwa and Davis (1995).

be in South Africa. It is not certain that folic acid fortification will reduce the risk for all population groups, e.g. Mexican Americans are at high risk and are apparently not amenable to folic acid supplementation (Suarez et al., 2000). The incidence of mycotoxicoses in South Africa is three- to six-fold higher in rural compared to urban blacks, but urban blacks have been reported, paradoxically, to have lower plasma folate concentrations than rural women (Ubbink et al., 1999). Household stored grain will not be fortified, further exacerbating the problem. The incidence of neural tube defects and folate status in rural and urban Africans need to be determined before the introduction of folic acid fortification.

### 3.6.2. Potential biotechnological solutions and status

The solution to the problems caused by mycotoxins in staple foodstuffs in Africa is not regulation, but reduction of fungal infection and mycotoxin levels (Marasas, 2001a, b). The most promising approach is to provide innovative solutions through biotechnology. Various biotechnological approaches are available to deal with mycotoxin contamination of grain in Africa, and as none will be sufficiently effective, it may well be best to use a combination of measures. Both the conventional and novel approaches to mycotoxin management have been recently reviewed (Duvick, 2001; Munkvold, 2003), and an earlier review by Bhatnagar et al. (1995) deals exclusively with molecular approaches.

The first line of defense is the control of the vectors carrying the fungi that produce mycotoxins, both the stem borers that cause the systemic infection of endophytic *Fusarium* spp., and the grain weevils, and especially in Africa the lepidopteran earborer (Setamou et al., 1998; Cardwell et al., 2000) carry the *Aspergillus* spp. The second line of defense is to suppress fungal attack, either by biocontrol or by engineering resistance to the fungi that produce the mycotoxins. The third line of defense against mycotoxins is to prevent their biosynthesis, and a fourth line is to degrade them in

the grain before they enter the food chain. These lines of defense are described below.

3.6.2.1. Excluding the vectors. Genetic engineering of insecticidal proteins has been shown to accomplish this in maize. Transgenic maize expressing the Cry1Ab protein has been shown to have half the fumonisin levels in conventional maize where insect pest pressure is significant (Munkvold, 2003; Munkvold et al., 1999). Mycotoxin levels also are highly dependent upon environmental factors such as humidity, and high humidity may exacerbate a low level of Bt-induced insect infestation. Studies in Canada and Europe did not show great reductions in fumonisin levels (Munkvold, 2003), but again the control levels were also low in these northern climes. However, a large difference, indicative of the African situation comes from experiments where Bt and non-Bt maize were artificially inoculated with corn borer. The fumonisin levels in the non-Bt maize were exceedingly high, and in the Bt maize 10-fold lower. Similar reductions in fumonisins were found in Bt maize in the warmer climes Spain and France, where the Bt maize always had less than  $0.5 \,\mu\text{g/g}$  and the normal maize had levels as high as 10 µg/g. The results with Bt maize and aflatoxin were not as consistently good, even in warmer climates (Munkvold, 2003). This perhaps due to the continued growth and toxin production in warm humid storage of the *Aspergillus*, whereas the fumonisin producing Fusarium spp. stop toxin production at harvest.

The use of biotechnology to control these insect vectors carrying mycotoxin-producing fungi is described in Sections 3.4.2 and 3.5.2, but a 90% reduction of fumonisins from a high level may not be sufficient in Africa, where maize is a large part of the diet. Thus the engineering of Cry proteins into maize represents a partial solution to the mycotoxin problem, and other approaches must be employed simultaneously if mycotoxin levels are to be significantly reduced in all locations. Plants will have to be engineered with stacked Bt genes, with separate specificities to

<sup>&</sup>lt;sup>a</sup> Australian dollars.

coleopteran and lepidopteran vectors of pathogens producing mycotoxins.

3.6.2.2. Controlling mycotoxin producing fungi. Biotechnology has been used to control fungi. Two forms of biocontrol have been tested in general. The first has been to find and then enhance the activity of mycopathogenic fungi. The many approaches for doing this are described in a recent book (Vurro et al., 2001). While this approach has been used to control various fungi, it is typically used to control them before they enter the plant, either in the soil or on the surface of plants. Such biocontrol agents have been engineered to degrade their host before entry, including organisms in the same genus. We are aware of no publications where this approach has been tested with the mycotoxin-producing pathogens. The fact that the activity of such mycopathogenic agents must be in the plant because of inoculation through the lesions caused by insect vectors may not be important. In the past, researchers have had some success using organisms related to the fumonisin and aflatoxin toxigenic fungi to compete with them, though not to control them (Cotty and Bhatnagar, 1994; Desjardins and Plattner, 2000; Dorner et al., 1999; Jardine and Leslie, 1999). The non-producing strains did have a modicum of fumonisin or aflatoxin reduction, but these are still organisms living at the expense of the crop plants, reducing yield.

The advantage of biocontrol agents is that they can typically be used on a large number of crop species; the approaches of engineering crops directly are variety specific, although it is possible that a construct used to engineer one crop variety or species can be used successfully with most others, but transformation is still not trivial, and many transformants must be made in order to ultimately choose one.

3.6.2.3. Controlling toxigenic fungal growth in planta. - Plants have been engineered with a large coterie of antifungal agents to prevent fungal growth such as phytoalexins (e.g. stilbene) and enzymes (e.g. chitinases and glucanases). To the best of our knowledge this strategy has rarely been used to prevent attack by toxigenic fungi. An amylase inhibitor from a legume inhibits fungal growth and aflatoxin production (Fakhoury and Woloshuk, 2001), so that gene is a 'candidate' for engineering suppression of Aspergillus.

3.6.2.4. Preventing mycotoxin synthesis and degrading mycotoxins in planta. The approach of preventing mycotoxin synthesis has been discussed for nearly a decade. This could be done by preventing the plant from producing (the elusive) signals that initiate toxin production in the fungi. A strategy further along has been to interfere with the metabolic pathways of mycotoxin production. To do this, the pathway had to

be elucidated and the genes cloned (Brown et al., 1999; Silva et al., 1996; Yu et al., 2000a, b). How precisely this information will be used is an open question.

The degradation of mycotoxins seems to be the route being investigated by a number of private enterprises, based on the number of recent patents issued. An esterase that degrades fumonisins has been isolated and cloned from a black yeast (Blackwell et al., 1999; Duvick et al., 2001) and other microorganisms (Duvick et al., 1998). Unfortunately, the patents claim that transgenic plants degrading fumonisins "can be made", and not that they have been made.

Two genes have been isolated for aflatoxin degradation. They were initially found epidemiologically in humans. The genetic susceptibility to cancer from aflatoxin was traced to a lack of either a glutathione transferase or an epoxide hydrolase that degrade aflatoxin due to a mutation in the genes encoding either of them (McGlynn et al., 1995). This led another group to find a human aflatoxin aldehyde reductase gene (Bandman et al., 2003). In an interesting patent application they propose its use only for therapy in humans and not for degrading aflatoxin at the source. In another patent application, Subramanian (2002) proposes to use gene shuffling to enhance the mycotoxin degrading activity of a number of mycotoxins, including aflatoxins and fumonisins. Thus, there seem to be a number of patented paths to mycotoxin degradation, but hard data are sparse to suggest which path may actually work.

### 4. Concluding remarks

The data in the previous sections quantify some of the most important biotic constraints to crop production in Africa. This leads to major questions:

- 1. are all the constraints recognized by the countries involved, and have they been given priority commensurate with the problem? We will see below that most are recognized but some are not;
- 2. are there similar problems elsewhere in the world and are they being addressed through biotechnology, if so, does biotechnology have an answer already? We will see that the problems exist elsewhere, but solutions currently exist only for the problems that are also economically acute in the developed world; and
- 3. how can the international community assist the African community in dealing with these constraints, especially those that have fallen through the cracks, i.e. dealing with the constraints that are not recognized at the policy making level and/or those that do not have "off the shelf" biotechnological solutions?

### 4.1. Validation based on national priorities

To determine how the agricultural constraints identified above by African scientists match with established national research priorities, the priorities and capacities of different African countries with respect to biotechnology were surveyed. Comprehensive assessments were obtained for Burkina Faso, Egypt, Ethiopia, Ghana, Kenya, Malawi, Namibia, Nigeria, South Africa, Tanzania and Uganda, and a limited amount of information for Morocco and Tunisia. This information will be presented in detail elsewhere, but relevant portions are summarized below.

Some of the identified constraints clearly do match with national research priorities. Specifically, most eastern African nations (particularly Kenya, Malawi and Tanzania), along with Egypt and South Africa, regard maize stem borers as one of their highest research priorities. Most of these same countries also regard parasitic weeds as a major agricultural problem. However, few countries have chosen to focus on grain quality issues such as storage pests and mycotoxins, though Namibia identified storage losses as a whole as requiring attention and Kenya has initiated some projects in this area. None of the countries proposed dealing with any but their major crops; none had as a priority to biotechnologically upgrade abandoned crops to increase crop biodiversity.

Thus, the processes used for prioritizing agricultural research needs do appear to overlook or downgrade a few critical problems. One of the reasons for this may be the lack of research capacity in the relevant areas; with respect to biotechnology, technical capacity was mentioned as a limiting factor at the national level in almost all of the nations surveyed. Most importantly, while all of these countries have some work ongoing with tissue culture propagation, most have no facilities or capacity

for genetic engineering. Thus regional collaboration and/or assistance by international NGOs and technical cooperation agencies will be needed to successfully address these agricultural constraints.

The surveys of the national priorities and capacities of different African countries with respect to biotechnology have demonstrated that technical capacity must be enhanced to reach the national goals in almost all cases.

### 4.2. Who is addressing what?

In a few cases the constraints are known, recognized at the political level, and are being addressed biotechnologically in Africa and in the world (Table 13). These cases include parasitic weeds and stem borers. Even there, the issues in the field are being sporadically addressed. Only maize is being addressed vis a vis *Striga*, and only by using a biotechnologically derived mutant. The available transgenic solutions, which are probably more durable, are not being put forward. Considerable work is being done with Bt maize, but as with Striga, sorghum and millet are not being addressed, which may result in an over reliance on maize. Transgenic answers to grass weeds in wheat are available in North America, but no answer is available for barley, a crop too often ignored. This results in over reliance on wheat, including using it for animal feed, despite barley being an excellent rotational crop that is more competitive with weeds than wheat, and is more stress-tolerant.

*Bemesia* and the virus it harbors are worldwide problems in vegetables, yet the chemical fix is apparently more attractive than biotechnology for commercial reasons (despite the rapid evolution of insecticide resistance), and to others who eschew biotechnology.

The problems that have huge impact on grain quantity (weevils) and quality (mycotoxins) are well known to the experts dealing with grain, and are both

Table 13
Pinpointed major intractable biotic constraints amenable to biotech intervention in Africa

Constraint	Type of genes or technology needed	Genes are known that will potentially overcome	Being addressed <sup>a</sup>		Commercialized	
			Outside Africa	In Africa	Outside Africa	In Africa
Grass weeds in wheat	Herbicide resistances	Yes	Insufficiently	No	Some	No
Parasitic weeds	Herbicide resistances	Yes	Research level	One case	No	One case
	Enhanced biocontrol	Some	Research level	No	No	No
Stem borer/maize	Bt	Yes, not perfect	Yes	Yes	Yes	One case
Stem borer/sorghum,	Bt	Yes, not perfect	No	No	No	No
Grain weevils	Bt type gene	Not clear	No	No	No	No
	Classical biocontrol	Fungi known	No	Yes	No	No
Bemesia/TLCV	Anti insect	No	No	No	No	No
	Anti virus	Insufficient	No	No	No	No
Mycotoxins	Suppress vector-Bt	Yes, not perfect	Yes	Yes	Yes	One case
	Suppress fungus	No	No	No	No	No
	Degrade toxin	No	No	No	No	No

<sup>&</sup>lt;sup>a</sup> A negative answer means that no group is known to be addressing the problem.

worldwide tropical problems. Their impacts were largely unaddressed by the biotechnology policy makers who generate national priorities as well as by the international donor and biotechnology communities. It is hoped that this report rectifies this situation.

One biotic constraint to crop production was discussed at the workshop but is not discussed above, because it is not in the area of crop protection. This is the poor availability of carbohydrate polymers from grain straw or stover to ruminant animals. This is because lignin sterically hinders the degradation of cellulose and hemicellulose by ruminant enzymes. Lignin content and composition can be and has been transgenically modified in other species, but not in grain crops. Slightly changing lignin content and adding urea to the straw before feeding could allow Africans to keep an additional 170 million goats per year on stover alone with a concomitant improvement of nutrition from milk and meat (Gressel and Zilberstein, 2003). No research group, to the best of our knowledge is addressing the transgenic modification of grain straws, although some research is being performed on "brown-midrib" maize, with mutationally modified lignin for silage (e.g. not grain) (Provan et al., 1997).

### 4.3. Outcomes from the compiled information

Several conclusions can be drawn from this analysis:

- the impacts of weeds, pathogens and pests on agricultural productivity in Africa cannot be perfectly quantified, yet it is clear the problems are great and priorities for biotechnological involvement can be set. There are several reasons for this lack of quantitative data, including the inherent difficulty in quantifying impacts in smallholder agriculture and fragmented markets, the lack of systems to analyze any existing data and, last but not least, the compounded effects of disease and pests not only on productivity but often on human and animal health and social cohesion;
- national and international science and technology assistance programs have had, in some areas, notable successes but these are few and far between when juxtaposed against the magnitude and multitude of problems that confront the continent; and
- the adversity of impacts of weeds, pests and disease on agricultural economies at the local and regional levels is magnified further by social, institutional and market failures. The analysis makes it abundantly clear that there are several pressing needs that still remain unaddressed.

The above conclusions beg the question: there are available technologies that can effectively address many of these priorities, but under what conditions? By looking at the whole spectrum of available technology

options it became clear that the new generation of "biotechnologies" could provide effective solutions to some of the most acute problems facing food security in Africa. However, in order to capitalize on such technological opportunities, the region must access three intellectual assets, namely genetic transformation technologies, elite germplasm, and useful genes.

These assets are mutually complementary and increasingly being covered by strong proprietary regimes. Even if they were to be made available by their respective owners for solving particularly acute problems there would still be considerable need for technological adaptation to specific ecological niches and farming situations. This, in turn, requires significant financial resources and stewardship mechanisms beyond the means of most developing countries and international funding and/or technology assistance agencies. To this end. The African Biotechnology Stakeholders Forums (ABSF) was formed as a not-for-profit, apolitical platform for sharing, debating and understanding all issues pertaining to biotechnology in agriculture, health, industry and environment. ABSF represents all stakeholders in biotechnology in Africa and has as its objectives to: improve public understanding of biotechnology through provision of accurate and balanced information; to explore innovative and appropriate biotechnology applications and facilitate their adoption and use; to create the capacity for information generation, dissemination and the wise use of biotechnology and to facilitate research, development, education and training on biotechnology as well as policy and infrastructure development for meeting Africa's needs in biotechnology.

The dearth of financial and institutional resources to address pressing problems in agriculture make the role of international development agencies pivotal in catalyzing effective partnerships amongst those who need the technologies, their respective owners, the national and international research centres in the region, and the end users. Numerous authoritative international bodies recognize that the adversity of the current situation in Africa requires intensification of efforts and greater participation of both the private sector and the international funding community. One practical initiative that, in the view of the authors, could be of great benefit for the region is the establishment of an International Biotechnology Trust that could be used to provide financing for the development of target oriented research or adaptation of existing proprietary technologies to the specific needs of countries seeking their acquisition.

The Trust would essentially constitute a mechanism to access the pool of available technologies for use in the developing world under preferential terms. The Trust would ensure that technology donors retain all legitimate rights with regard to their respective innovations.

The establishment of the Trust would provide developing countries with a unique resource to:

- access proprietary technology through licensing under preferential terms;
- engage in technology adaptation thus increasing their respective capacities; and
- acquire and strengthen capacities in technology intelligence and regulatory oversight (IP and biosafety).

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