CHAPTER 11. *FUSARIUM OXYSPORUM* F.SP. *STRIGA*, ATHLETES FOOT OR ACHILLES HEEL?

SHORT TITLE: STRIGA'S ADVERSARY

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Abstract. Parasitic weeds are major contributors to hunger, malnutrition, and food insecurity across sub-Saharan and northern Africa by reducing crop yields in half. Over twenty million hectares of cereal grains in sub-Saharan Africa are infested with *Striga* (witchweed). Food production losses due to *Striga* in African countries range from 20% to 90%, amounting to over 10 million tons of food lost annually. The control options for *Striga* are currently ineffective and management possibilities for these weeds are urgently needed. The research progress with a specific

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forma speciales of *Fusarium oxysporum* as a biological control for *Striga* in Africa illustrates the potential to positively impact many lives and improve the health and livelihood of rural and urban poor. Can *F. oxysporum* wild type be the Achilles heel of *Striga*, or do we need enhanced biocontrol to achieve rapid, safe, cost-effective solutions for this major biotic constraint to food production in Africa?

Keywords: witchweed, chlamydospores, seed coating, rhizosphere competence, hypervirulence

1. Introduction

Parasitic weeds, the scourge of African farmers, are major intractable biotic constraints to food production in Africa.[1] *Striga* spp. (witchweeds) are obligate parasitic weeds that parasitize the roots of cereal crops and food legumes. After attachment to the crop hosts' roots, they penetrate into the vascular system of the crop, removing water, photosynthates and minerals. Parasitic weeds are major contributors to hunger, malnutrition, and food insecurity across sub-Saharan by having yields in major crops in infested areas. Striga infests 26 million hectares in sub-Saharan Africa. The control options for *Striga* are currently ineffective and novel management strategies for Striga suppression are urgently needed. The development of biological control for *Striga* in Africa illustrates the potential to impact many lives and improve the security of struggling regions. There is a need to ascertain whether biotechnologies can supply rapid, safe, cost-effective solutions to these intractable biotic constraints. Thus, for sustained *Striga* control and management, it is imperative to foster new integrated approaches including biotechnological solutions, with rigorous resource mobilization, wider strategic partnerships, novel multidisciplinary linkages and participatory approaches with farmers.[1]

2. Striga in sub-Saharan Africa

The genus *Striga* (Orobancaceae) contains several obligate hemi-parasitic flowering weeds that are major biotic constraints to cereal and legume production in sub-Saharan Africa (SSA). *Striga* species are hemi-parasites, photosynthesizing about 20% of their needs after emerging from the soil. *Striga hermonthica*, *S. asiatica*, and *S. forbesii* parasitize cereal grain crops, sorghum, millets, maize and upland rice while *S. gesnerioides* parasitizes legume crops, mainly cowpeas. *Striga* species have become a scourge to

cereal production and legumes where fertility is low and water/rainfall is low or erratic. The genus is most widespread in western Africa where it infests 17 million hectares or covers 64% of the cereal production area with a potential coverage of almost 100% in the semi-arid and sub-humid tropical zones[1]. In eastern and central Africa, *Striga* infests 3 million hectares (23% of cereal area and 1.6 million hectares in southern Africa (mostly in Mozambique) are infested. 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) (Table 1)

Table 1. Sub-Saharan Africa countries with most *Striga* incidence/infestation

Country	*Striga* infested area Sorghum and Millet[a]		Maize	
	('000 ha)	% total	('000 ha)	% total
Botswana	30	30	2	10
Burkina Faso	1318	50	26	10
Eritrea	64	40	0	0
Ethiopia	528	30	80	5
Kenya	80	53	225	15
Mali	1513	70	20	10
Mozambique	150	40	122	10
Niger	4989	70	-	-
Nigeria	8720	80	904	22
Senegal	411	40	3	0.05
Sudan	1875	30	17	10
Tanzania	650	90	214	12
Total/mean	20,330	56	1613	15

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

* includes finger millets in the lake zone of east central Africa.

[a] Includes both sorghum and pearl millet combined in West African countries only. Source: Modified from Gressel et al. [1]

In many places in Africa, the *Striga* problem has reached epidemic proportions with the situation being worst in subsistence agriculture. Yield losses from damage by *Striga* are often very significant, ranging from 10% to 70%, depending on the crop cultivar, degree of infestation, rainfall pattern and soil degradation, and estimated at 40% on average.[2] Food production losses due to *Striga* in the SSA countries range from 20% to 90% (Table 2), amounting to over 8 million tons of food lost annually[1]. 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. Crop rotation is probably one of the most effective ways to reduce *Striga* infestations and increase maize yields and income considering the limited resource base of small-scale subsistence farmers in SSA.[3] Yet most of the rotational crops (forage legumes) do not provide the food needed to sustain the farm families. Land use intensification and increasing cereal mono-cropping, with little or no use of purchased external inputs, have contributed immensely to exacerbate the *S. hermonthica* problem in Africa. The farmers' plight has been compounded by the environmental and policy factors that fostered *Striga* spread.

Table 2. Sub-Saharan Africa countries with the highest food production losses due to *Striga**

Country	Estimated yield loss* (%)	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	930-1,160
Nigeria	35	3,750
Sudan	30	1,230
Tanzania	up to 90	550
Togo	35	70
Total/mean	39-45	8,110-8,520

* Loss includes sorghum, millets, and maize. Compiled by A.B. Obilana, from NARS documents, reports and personal records. Source: Gressel et al.[1]

3. *Striga hermonthica*

Striga hermonthica, the most economically important parasitic seed plant in the world[4], is endemic in the African savanna and the Sahel where it devastates the yields of maize, sorghum, millet, and rice, the major staple foods for over 300 million people in SSA. Annual crop losses in cereals caused by *S. hermonthica* vary from about 10% (at low levels of infestation) to complete crop loss and total abandonment of cereal production in severely infested fields. It causes an annual loss of about US$9 billion. Recent surveys have abundantly confirmed that farmers in

these areas urgently and desperately need effective, inexpensive and sustainable control options as components of an integrated *Striga* management (ISM package).

Numerous techniques exist for the management of *Striga*. Each technique has value in certain situations, and limitations in others. For example, a new technique using herbicide treatment of maize seed of a herbicide-resistant maize is highly successful[5] and has been commercialized, but only for east-African short season maize, while it is now being developed for longer season maize, but not for other crops attacked by *Striga.* In many cases, valuable techniques are unavailable to the subsistence farmers who need them the most. The greatest deficiencies in the needs of subsistence farmers are short-term techniques that will enable the effective production of susceptible crops in *Striga* infested land. Techniques that will protect crops from parasitism by *Striga*, and provide remedial control of *Striga* are urgently needed. 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, and novel multidisciplinary linkages in participation with farmers. One potential option, that obviates some of the problems of several of the other options, is the use of *Fusarium oxysporum* f. sp. *striga* for the biological control of *S. hermonthica*. This solution would be applicable to all varieties of all crops attacked by *Striga.*

4. Biological control of *Striga hermonthica*

Both insects and fungi have been proposed for biocontrol of Striga. 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 while having little yield promotion.[6] Various fungi have been tested both for pathogenicity on *Striga* but none are yet in wide scale field testing.

Fusarium species are the most prevalent fungi associated with diseased *Striga* plants. Controlled environment chamber evaluation of 81 fungal isolates from three countries (Burkina Faso, Mali and Niger) found an isolate of *Fusarium oxysporum* from Mali (isolate M12-4A), grown on sorghum straw and incorporated into pots, that successfully prevented emergence of *S. hermonthica*. This resulted in a four-fold increase of sorghum dry matter.[7] Subsequent evaluation of efficacy of the M12-4A isolate in the field in Mali, using chopped or ground sorghum straw inoculum, resulted in 60% reduction of emerged *Striga* at 82 days after sowing, while sorghum biomass was doubled[8] compared with the control.

Further work with isolate M12-4A has reported complete inhibition of *S. hermonthica* emergence when the fungal spore (chlamydospore) powder was added to the soil with sorghum seeds or by sowing sorghum seeds that were also coated[9] with the chlamydospores. Chlamydospore powder treatments reduced *S. hermonthica* emergence by 78 to 92 % (Table 3). In related studies from Nigeria and Burkina Faso, other isolates of *F. oxysporum* (PSM197, 4-3-B) inhibited *Striga* seed germination and reduced the number of emerged *S. hermonthica* plants in pot[10] and field[11,12] trials.

Table 3. Effect of *Fusarium oxysporum* M12-4A on *Striga hermonthica* emergence in the field.

Inoculum treatments per seed pocket	Striga plants / plot		
Control (no straw incorporated)	32.1[1]	(17.3)[2]	a[3]
Sterilized straw control (10g)	16.8	(6.2)	ab
Sterilized ground straw control (2.6g)	21.3	(12.5)	ab
Solid substrate ground inoculum (2.6g)	7.9	(4.5)	b
Chlamydospore powder (0.5g)	6.9	(4.9)	b
Chlamydospore powder (0.5g) + sterilized straw (10 g)	3.6	(1.9)	b
Chlamydospore powder (1.0g)	2.7	(1.7)	b
Chlamydospore powder (1.0g) + sterilized straw (10 g)	2.5	(1.4)	b

[1]Mean number of *S. hermonthica* in plots

[2]Values in parentheses are standard errors.

[3]Values having the same letter are not significantly different at = 0.05 according to the Student-Neuman-Keuls multiple range test.

Source: Ciotola et al[11].

F. oxysporum f. sp. *striga* is host limited. Several crop species (sorghum, pearl millet, maize, rice, fonio, cotton, groundnut, cowpea and okra) were immune to isolate M12-4A[7]. These and other crops are also immune to isolates from Ghana, Sudan, and Nigeria.[13,14] All *S. hermonthica* isolates of *F. oxysporum* f. sp. *striga* are pathogenic only to *S. hermonthica*, and possibly *S. asiatica*[14]. Isolate M12-4A does not produce mycotoxins under all conditions tested, and hence it does not constitute a known health hazard to humans or livestock[15].

Mass production and delivery of the biocontrol agent to its target are critical phases in biocontrol projects. Techniques that have been suggested for mass production of *F. oxysporum* inoculum include on-farm models, cottage-industry models and small entrepreneur industry models. *Fusarium* can be grown on a range of cheap, crude agricultural products, including sorghum stubble. Several methods for mass production of the fungus on sterilized sorghum straw have been developed.[8,9] Effective biological control of *S. hermonthica* with M12-4A was achieved with inoculum

produced using a simple fermentation system with sorghum straw as the growth substrate for inocula. Sorghum seeds were coated with inoculum using gum arabic as the adhesive for inoculum delivery at farmers' fields in researcher-managed trials.[9] When applied as a seed coat, only 80g of the chlamydospore powder are required per hectare. To facilitate broad usage of the *F. oxysporum* isolate M12-4A, an inoculum production strategy based on cottage industry model was suggested that utilizes a liquid fermentation process and inexpensive locally available substrates (including sorghum straw and gum arabic).[11] Four villages in Mali participated in 2000 in liquid mass production of M12-4A in cooking pots and in coating seed. Seed coating activities were highly successful, but all production vessels became contaminated and no viable inoculum was produced.[16] Other, more rigourous production systems need to be critically evaluated.

In addition to the above powder formulation, inocula have been applied directly into the seeding holes and several granular formulations, including sodium-alginate and wheat flour-kaolin "Pesta" granules have been evaluated.[17]

5. Molecular Characterization of *F. oxysporum* Wild Types

The genetic diversity among the various isolates of *Fusarium oxysporum* from *Striga hermonthica* has indicated a high degree of genetic similarity (Ciotola *et al*. unpublished). Vegetative compatibilities of 14 isolates of *F. oxysporum* from diseased *S. hermonthica* were determined using nitrate non-utilizing mutants. All *F. oxysporum* f.sp. s*triga* collected from Mali, Niger and Kenya were in one vegetative compatibility group (VCG) and thus genetically similar. Random amplified polymorphic DNA assays were carried out on a large range of isolates of *Fusarium oxysporum* to identify markers only common to *F. oxysporum* strains isolated from *Striga*. One fragment of 3500 bp was cloned and used to probe Southern blots of DNA from *Fusarium oxysporum* isolates as well as various heterogeneous organisms and plant tissue. The fragment hybridised only to DNA from *Striga* isolates and two *F. oxysporum* isolates that originated from sorghum. The amplified product (600 bp) was sequenced and two pairs of SCAR (sequence characterised amplified region) primers (M12-4A/R and M12-4A/F) were generated for use in polymerase chain reaction (PCR). One fragment of 600bp was generated following PCR of all *F. oxysporum* f. sp. s*triga* isolates and from one *F. oxysporum* from sorghum. Two new SCAR primers (FUN001 and FUN002) were designed containing the most sequence differences between the target isolate (M12-4A) and the sorghum *F. oxysporum* isolate O-1202 and tested in conventional PCR assays. FUN001 and FUN002 amplified only one band of 157 bp in all isolates

from *Striga*. No amplified product was detected in the sorghum *F. oxysporum* isolate. The same primers were used in real-time PCR assays to reconfirm their specificity and determine their sensitivity detection level. PCR assays confirmed the VCG results indicating *F. oxysporum* isolates from *Striga* from west and east Africa are genetically similar suggesting co-existence of *F. oxysporum* f. sp. *striga* with its host across SSA.

6. Enhancement of Fusarium oxysporum f. sp striga?

Different *F. oxysporum* isolates have reduced *S. hermonthica* by 40 to 100% in laboratory, pot and field trials. However, extensive field trials to ascertain field efficacy of *F. oxysporum* to control *S. hermonthica* have not yet been conducted. Will the *F. oxysporum* wild type be sufficiently virulent and competent to achieve the desired level of *Striga* reduction? The *Striga* problem in Africa is critical and it behooves us to examine all means to find a solution for this problem. Perhaps one or more of the following biotechnological approaches may improve the virulence, deployment, and success of *F. oxysporum*.

6.1. OVER EXPRESSION OF AMINO ACIDS

Amino acid toxicity has long been observed in plants, with different amino acids effecting different species of plants. It is not surprising then that single amino acids, when applied externally to a plant, can inhibit plant growth and development.[18] Examples are the severe seedling inhibitions when valine is applied to germinating seeds of *Papaver somniferum* and *Cannabis sativa*, methionine inhibition of *Cirsium arvense*, and lysine inhibition of *Centaurea diffusa*. These amino acid inhibitions can be as a result of direct application of specific amino acids to the soil, or by plant pathogens that excrete unusually high amounts of these amino acids.[19] Recently, Vurro et al.[20] have reported that 2 mM methionine was able to almost completely inhibit the germination of *Orobanche ramose*, a related parasitic weed. When methionine was applied to tomato roots, the number of developing tubercles of the parasite was reduced. Preliminary work indicated that *Striga* was sensitive to leucine, threonine and tyrosine.

6.2. GENERATING TRANSGENIC HYPERVIRULENT FUSARIUM STAINS

Several strains of *F. oxysporum* and *F. arthrosporioides* that attack *Orobanche* spp.[21] 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, doubling virulence[21], although this is still far less efficacy than farmers need. Far stronger toxic genes are needed to enhance virulence, and the NEP1 gene, used to enhance a different mycoherbicide[22] also was active in enhancing the virulence of a *F. arthrosporioides* that is specific to *Orobanche* spp. (Chapter 16).

A variety of hypervirulence genes are being transforming into two strains of *Fusarium* that attack *Orobanche.* These same constructs could be transformed into the *Fusarium* isolates used as biocontrol agents against *Striga*. The biosafety aspects of using transgenically hypervirulent biocontrol agents are specifically addressed in references 23, 24 and Chapter 19.

6.3. TECHNIQUES FOR OPTIMAL APPLICATION OF THE BIOCONTROL

The current state of the art is to apply the *Fusarium* biocontrol agent as a seed dressing using gum arabic[9] as a sticker. Alternative approaches may include various granular or pelletized formulations placed in the planting hole or applied during weeding operations. The *Striga* infestations in Africa cover vast areas and the idea of aerial dispersal[25] and soil penetration on seed is most intriguing. One suggestion is to deliver the biocontrol agent on the seed of a non-host reclamation plant species. In this method, it is hypothesized that the biocontrol agent could saprophytically colonize the roots of the seedlings of the reclamation plant as it establishes and take up residence in the soil profile where it could then come in contact with *Striga*. Selection of the carrier plant species will be an interesting challenge.

6.4. RHIZOSPHERE COMPETENCE AND PERSISTENCE.

The biology of *Fusarium* spp. in the soil, root, and rhizosphere is extremely varied. *Fusarium* spp. can be persistent in the soil as saprophytes, can develop large amounts of mycelium on the rhizoplane and in the rhizosphere, and can invade root epidermal and cortical tissues either pathogenically or non-pathogenically.[26] The activity of *F. oxysporum striga* isolates in each of these regards is not fully understood.

How far will *F. oxysporum* f. sp. *striga* move through the soil/rhizosphere? How long will *F. oxysporum* persist in the rhizosphere? What level of rhizosphere colonization is required for effective control of *S. hermonthica*? How is the biology of *F. oxysporum* in the rhizosphere affected by abiotic and biotic factors? How will selected and transformed

strains respond? Answers to these questions will be critical for designing the most effective strategies for the deployment of *F. oxysporum*.

6.5. COMPARE BIOLOGICAL EFFICACY OF THE BICONTROL AGENT WITH CONTROL OPTIONS BEING PRACTISED BY FARMERS

Additional on farm field trials with several *F. oxysporum* isolates are presently ongoing in Benin and others are planned. These trials should help answer the question of virulence and biocontrol efficacy of *F. oxysporum.* Certainly, enhanced biocontrol would be an additional benefit in the struggle against *Striga*.

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