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Pathogens Attacking *Striga hermonthica* and Their Potential As Biological Control Agents

Abbasher Awad Abbasher and Joachim Sauerborn

University of Hohenheim (380), PO Box 700562, 7000 Stuttgart 70, FRG

Surveys were carried out in the Sudan for microorganisms pathogenic to *Striga hermonthica* with the objectives of their isolation, identification and to explore their potential as biological control agents. Twenty-eight fungal and 2 bacterial species were isolated from infected *Striga* plants. Pathogenicity tests proved that many of the fungal species were pathogenic to *Striga*. Sixteen of these fungal species were reported for the first time from *S. hermonthica*. *Fusarium nygamai* and *F. semitectum* var. *majus* reduced the emergence of *Striga* plants up to 97 and 82% respectively, when mixed with soil preplanting. Using root chambers to observe underground stages of host and parasite it could be shown that *F. nygamai* reduced *Striga* incidence by >90%. As the results of *Striga* control and delayed emergence, the performance of sorghum was improved significantly.

Introduction

Striga hermonthica (Del.) Benth. (Scrophulariaceae) is widespread in irrigated and rainfed areas of the Sudan, it parasitizes sorghum, millet, maize, sugar-cane and a range of graminaceous species. The witchweed problem is especially severe in the rainfed mechanized schemes of Sudan, where sorghum and millet represent the main crops grown almost in a monocropping system. At this time *Striga* represents the largest biological constraint for the grain production in Africa. The area cultivated with cereals and actually infested by *Striga* is estimated at 21 million ha in Africa. The overall loss in grain production amounts to 4.1 million tons (Sauerborn 1991).

Less work has been done on microorganisms associated with *Striga* spp. compared to insects. No records of bacteria or virus have been traced but several authors have isolated fungi. Staples (1958, cited in Zummo 1977) reported the occurrence of *Cercospora* sp. and *Phoma* sp. on *S. asiatica* in Southern Rhodesia. Wager (1931) obtained *Pythium ultimum* in culture from *Striga* seeds. Naj Raj (1966) reported the occurrence of *Alternaria* sp., *Cercospora* sp., *Neottiospora* sp. and *Phoma* sp. on *S. asiatica* and *S. densiflora* from India. Meister and Eplee (1971) isolated 5 fungi, in North Carolina, which were found to be pathogenic to *Striga*. These include *Curvularia geniculata, Fusarium roseum, F. solani, Rhizoctonia solani,* and *Sclerotium rolfsii.* Zummo (1977) observed 3 diseases of *S. hermonthica* in West Africa, including a leaf spot, caused by an undescribed species of *Cercospora*, a vascular wilt caused by *F. equesti* and a stem lesion caused by a *Phoma* species.

The purpose of the present work was to survey for the native pathogens and to explore the feasibility of using them to control *Striga hermonthica.*

Methods and Materials

Collection of Infected Striga Plants

From August until the end of October 1989, sorghum fields were surveyed for fungi and bacteria associated with *S. hermonthica* in different areas in the Sudan. These areas included the irrigated scheme (Gezira and Managil) and the rainfed areas in east Sudan (Elgadarif) as well as in the southeast area (Blue

Nile). Plant pathogens were isolated from different parts of *Striga* plants that showed symptoms like lesions, rots, wilting, browning and from dead plants.

Isolation, Culturing and Identification of Fungi and Bacteria

Samples of diseased tissue were assayed for fungal pathogens by surface sterilization in 1% sodium hypochlorite, rinsing in sterile water and planting onto potato-dextrose agar (PDA) to which 2-bromo-2-nitropropan-1,3-diol (bronopol) was added to prevent bacterial growth. Pure cultures were prepared by single spore isolation or by hyphal tip isolation. Isolated bacteria were propagated onto nutrient agar (NA). Identification of fungi and bacteria was confirmed by the Deutsche Sammlung von Mikroorganismen (DSM), Braunschweig, FRG, the Commonwealth Mycological Institute (CMI), London, England, and through the Biologische Bundesanstalt für Land- und Forstwirtschaft, Instititut für Mikrobiologie, Berlin, FRG.

Pathogenicity Tests

In separate experiments, different fungi and bacteria, isolated from *Striga hermonthica*, were tested for their pathogenicity to *Striga* seeds and plants. All experiments were conducted in the glasshouse where the RH ranged 25-50% and the temperature between 35-22°C dependent on day or night time.

Pathogenicity to Striga *Plants*

Sorghum was grown in *Striga* infested soil using plastic pots (18 cm dia). Six to 8 wks after planting, when *Striga* plants were 5-15 cm tall, they were sprayed to run-off with spore suspensions from 3-4 wks old cultures grown on PDA or vegetable-juice agar (Miller 1955). After inoculation, plants were placed under dew chambers for 48 h. Pathogenicity of *Erysiphe cichoracearum* was conducted by use of natural spread of conidia from heavily infected *Striga* plants. *Fusarium* spp. as soil-borne pathogens were tested for pathogenicity by adding agar discs from Petri dishes containing large colonies of the isolates to the soil. Number of infected and killed *Striga* were recorded 2-3 wks after inoculation. Re-isolation was done for confirmation.

Pathogenicity of Fusarium *spp. to Seeds and Early Stages of* Striga

Ten different isolates of *Fusarium* spp. were evaluated for pathogenicity to *Striga hermonthica*. The fungi were propagated on vegetable-juice agar medium which were applied preplanting at a rate of 20 g/kg soil in pots (13 cm dia) containing a mixture of sterilized clay and sand (2:1 volume proportion). Five sorghum plants and 100 mg *Striga* seeds were sown/pot. After 2 wks, sorghum plants were thinned to 2 plants. Sorghum, with and without *Striga* seeds, but without fungus inoculum were kept as control. A randomized complete block design with 4 replicates was used. The experiments were repeated once.

For more detailed studies with *F. nygamai* (isolate 3003), which proved to be highly pathogenic to *Striga hermonthica*, the fungus was propagated on barley grains. The barley grains were filled in 2,000 ml wide mouth glass flasks and hydrated overnight, excess water was poured off and the medium was sterilized in the autoclave for 60 mins at 15 pounds pressure (120°C). After 24 h, each flask was inoculated with 10 ml spore suspension (approximately 1×10^6 spore/ml deionized water), prepared from 10-d-old cultures. The flasks were incubated at room temperature for 2 wks before they were used for inoculation. This experiment differs from the one previously described by taking 10 g of inoculum/kg soil using 18 cm dia pots with 5 replications. Experiments lasted for 3 months. Measurements were taken for *Striga* incidence (number of emerged *Striga* plants counted weekly), sorghum height (measured every 2 wks after the first *Striga* emergence), and dry weight of sorghum (shoot and panicle, determined after drying for 24 h at 120°C). Data were subjected to the analysis of variance at $P = 0.05$. Data included zero values, were transformed to $\sqrt{(x + 1)}$ (Little and Hills 1978).

Root Chamber Experiments

Since the isolate 3003 of *F. nygamai* proved most effective for *Striga* control this isolate was evaluated in root chambers to investigate the effect of *F. nygamai* on underground stages of *Striga*. Vessels were used as described by Linke and Vogt (1987). Wheat flour was evenly distributed on the surface of filter papers as nutritive medium for the fungus. Ten ml of mycelium and spore suspension (approximately $4x10^6$ spore/ml deionized water) prepared from 14-d-old fungal cultures was added to cover the whole surface of the filter paper before non-preconditioned seeds of *Striga* were sprinkled by hand. Control vessels were treated with deionized water instead of the mycelium and the spore suspension. Evaluation was done under a binocular microscope in 7-d intervals for 5 wks, counting the number of germinated *Striga* seeds, number of *Striga* attached to sorghum roots, number of *Striga* shoots and the number of germinated *Striga* which were killed. The experiment was repeated twice using 6 replications/experiment.

Host Range

The host range of *F. nygamai* (isolate 3003) was determined by testing 19 different crop species. The inoculum used was prepared on barley grains as described above and was applied preplanting at a rate of 10g/kg soil.

Results

Isolation and Pathogenicity Tests

Twenty-eight fungal species and 2 bacteria were isolated from the diseased, surface-sterilized *Striga* plants which had been collected from different sorghum production areas in the Sudan (Table 1). Twenty-four of the 28 fungal species and the 2 bacteria were screened for their reaction against *S. hermonthica*. Nineteen of the fungal species were found to be pathogenic to *Striga*. The 2 bacteria were found to be non-pathogenic (Table 1). Among the spores suspensions sprayed, *Phoma sorghina* proved to be highly pathogenic to *S. hermonthica*: 70% of the inoculated *Striga* plants were killed.

Infection of *P. sorghina* appeared on leaves and stems of *Striga* plants as lesions of variable extent resulting in its death. *P. sorghina* also infected the host plant, sorghum. *Erysiphe cichoracearum* infected all *Striga* plants subjected to the infection. Symptoms of powdery mildew appeared as white powdery patches covering leaves and stems. These patches are of variable size, severe infections result in complete cover of leaf and stem surface and in premature drying up of the leaves or the whole plant. The powdery mildew isolate did not infect sorghum plants.

Ten isolates of *Fusarium*, as soil-borne pathogens, were screened for their potential to reduce *Striga* emergence (Table 2). Two isolates of *F. nygamai* (3003 and 3004) and 1 isolate of *F. semitectum* var. *majus* (3002) reduced the total number of emerged *Striga* plants by 96, 93 and 82% respectively, while the other tested isolates gave little or no reduction in number of emerged *Striga* plants. Sorghum shoot dry weight increased significantly due to presence of *F. nygamai*.

In the second trial where *F. nygamai* was propagated on barley grains and applied at a rate of 10g/kg soil, it reduced emergence by 75%. A delay in the emergence of *Striga* plants was also noticed (Fig. 1). As a result of *Striga* control, performance of sorghum plants was improved. A highly significant increase in sorghum height (Fig. 2), sorghum shoot dry weight and yield was achieved (Table 3).

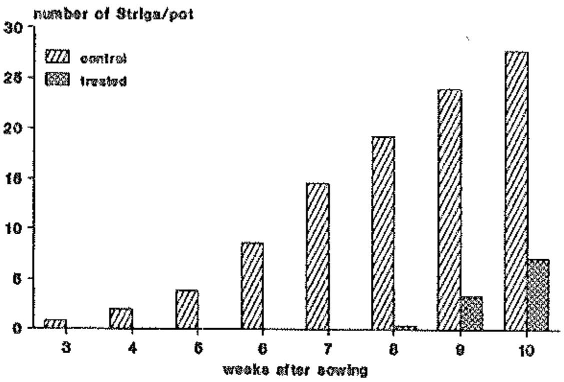

Figure 1. Number of *Striga hermonthica*/pot as influenced by *Fusarium nygamai*.

Table 1. Fungi and bacteria isolated from infected *Striga hermonthica*, collected from the Sudan, and their pathogenicity to *S. hermonthica*.

Isolate	Plant Part[1]	Pathogenicity to *S. hermonthica*[2]
Fungi		
Aspergillus flavus Link *ex* Fries	LStS	–
A. fumigatus Fresenius	LStS	–
A. niger van Tieghem	LStS	–
A. terreus Thom.	LStS	–
Alternaria alternata (Fr.) Keisser	LS	+
A. chlamydospora Mouchacca	L	+
A. longissima Deighton & MacGrovie	L	0
Bipolaris specifera (Bainier) Subram	LS	+
B. zeicola (Stout) Shoem	LS	+
Cercospora sp.	L	0
Curvularia cymbogenis (Dodge) Groves & Skolko	L	+
C. lunata (Wakker) Boedijin	LS	+
C. pallescens Boedijin	LS	+
Diplodia sp.	L	+
Drechslera gigantea Heald & Wolf	L	+
D. longirostorata (Subram.) Subram	LS	+
D. papendorfii (van der Aa) Ellis comb.	L	+
Exserohilum rostoratum (Drechsler) Leonard & Suggs	LSt	+
Erysiphe cichoracearum DC.	LSt	+
Fusarium chlamydosporum var. *chlamydosporum* Wollenw. et Reinking	LSt	–
F. equiseti (Corda) Sacc.	LStS	+
F. nygamai Burgess & Trimboli	LStS	+++
F. oxysporum Schlecht Snyd. & Hans	LSt	+
F. semitectum var. *majus* Wollenw.	LSt	+++
Phoma medicaginis Malbr. and Roum	LSt	+
P. sorghina (Sacc.) Boerema *et al.*	LStS	+++
Phythomyces sp.	LS	0
Rhizopus oryzae Went and Prinsen Geerlings	LStS	–
Bacteria		
Bacillus subtilis Ehrenberg	LStS	–
Pantoea agglomerans Ewing and Fife	LS	–

[1]L= Leaf, St. = Stem, S= Seeds.
[2]Pathogenicity: – = no reaction; + = 1-33% of *Striga* killed; ++ = 34-68% of *Striga* killed; +++ = 69-100% of *Striga* killed; 0 = not tested.

Root Chamber Experiments

In this experiment *F. nygamai* reduced the germination of *Striga* seeds, prevented germinated *Striga* to attach to sorghum roots to a great extent and reduced the number of *Striga* shoots 93, 94 and 96% respectively compared to the control (Fig. 3). The fungus, also killed 74% of the germinated *Striga* by attacking their germ tube. This was observed to change to dark brown colour and to die before attachment as a result of the infection. Other *Striga* plants were found to be killed after their attachment.

Host Range

Application of *F. nygamai* to the soil, preplanting, with 19 different crop species, indicated that *F. nygamai* is non-pathogenic to these crops (Table 4).

Discussion

The work presented the first attempt to control *Striga* with fungi in the Sudan. All the fungal species except *Aspergillus* spp., *Rhizopus oryzae*, and *Drechslera longirostorata* were reported for the first time in the Sudan. *P. sorghina* proved to be highly pathogenic to *S. hermonthica*. There are previous reports on *Phoma* spp. in Africa. In Zimbabwe, *Phoma* spp. were found to kill *Striga*. In Nigeria, *Phoma* spp., cause stunting but not the death of the plants. The fungus was found as a minor pathogen of *Sorghum and Setaria* species in the tropics (Punithaliangam and Holliday 1972). In the present work *Phoma* attacked sorghum.

E. cichoracearum infected all *Striga* plants subjected to the infection. *Sphaerotheca fuliginea*, another powdery mildew, has been isolated in India and Africa. In India it is recorded as causing premature drying up of *Striga densiflora*, and less often *S. asiatica* (Naj Raj 1966). Powdery mildew fungi are obligate pathogens and usually are not severely damaging but weaken the plant and possibly reduce its seed production. Host-specific *forma speciales* are known crop pests so that strains attacking *Striga* spp. probably exist. Powdery mildew infestations tend to be more severe at low humidities and they can be potential useful biological control agents in semi-arid zones (Greathead 1984).

Table 2. Reduction of *Striga hermonthica* and improvement of sorghum shoot dry weight (g). as influenced by different *Fusarium* isolates. Means within the row differ significantly at *P*= 0.05.

Tested Fungi	No. of *Striga*/pot		%	Sorghum Shoot Dry Wt		% Im-
	Control	Treated	Reduction	Control	Treated	provement
Fusarium sp. isolate 3001	14.8	14.3	3.8	8.55	5.43	0
F. semitectum var. *majus* isolate 3002	7.5	1.3	82.7	9.60	11.60	21
F. nygamai isolate 3003	7.5	0.3	96.0	9.60	18.75[1]	95
F. nygamai isolate 3004	7.5	0.5	93.0	9.60	16.98[1]	77
F. semitectum var. *majus* isolate 3005	7.5	14.5	0	9.60	7.03	0
F. chlamydosporum var. *chlamydosporum* isolate 3006	14.8	14.8	0	8.55	10.00	17
F. equesti isolate 3007	14.8	15.5	0	8.55	7.30	0
F. equesti isolate 3008	14.8	17.3	0	8.55	12.10	40
F. equesti isolate 3010	11.3	15.3	0	8.75	8.22	0
F. oxysporum isolate 3011	13.0	8.8	32.0	7.63	5.58	0

[1]Means within the row differ significantly at *P*= 0.05.

F. nygamai and *F. semitectum* var. *majus* reduce the emergence of *Striga* plants up to 96 and 82% respectively. A delay of *Striga* emergence was also observed. The *Fusarium* species, as soil-borne pathogens, could probably be of use as natural antagonists for parasitic weeds. *F. oxysporum* f. sp. *orthoceras* was found to control >90% of the parasitic weed, *Orobanche cumana* Wall. on sunflower in Bulgaria (Bedi and Donchev 1991). In Iran, *F. oxysporum* decreased the broomrape up to 75% and increased the yield of tobacco up to 80% in field trials (Mazaheri *et al.* 1991). Increase of sorghum height, sorghum shoot dry weight and panicle was obtained when *F. nygamai* was applied preplanting of sorghum and *Striga*. This is mainly due to reductions in germination and attachment of *Striga*, resulting in lower numbers of their emergence. The fungus attacked the germ tube of the parasite, attached *Striga* were

Table 3. Effect of *Fusarium nygamai* on the incidence of *Striga hermonthica* and performance of sorghum.

	Striga		Sorghum	
Treatment[1]	No. Shoots/Pot	Shoot Height (cm)	Shoot Dry Wt. (g)	Panicle Dry Wt. (g)[2]
Control (–)	–	149.0	40.1	2.9
Control (+)	27.8	64.0	2.3	1.0
F. nygamai	7.2	150.0	50.0	2.9
S.E.		11.2	2.3	0.2

[1]Control: (–) = without *Striga*; (+) = with *Striga*.
[2]Transformed $\sqrt{(x+1)}$

also infected. Emerged *Striga* plants were found to be rarely infected. These results were similar to findings of Bedi and Donchev (1991). They found that *F. oxysporum* infected the soft tissue of the radicle of the parasite *Orobanche cumana* and killed it before it could attach to the host root. Pathogenicity tests with *F. oxysporum* f. sp. *orthoceras* showed that broomrape is susceptible to the fungus attack at all stages of its growth. Infection may take place from tubercle to flowering stage. However it is not known if *F. oxysporum* infects the broomrape seeds. In the root chamber trials it was shown that *F. nygamai* infected *Striga* seeds and reduced their germination by 93%.

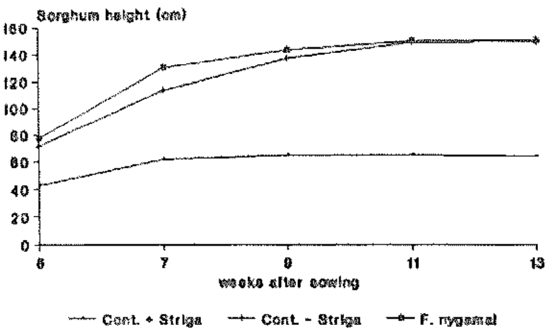

Figure 2. Increase in sorghum height as influenced by *Fusarium nygamai* in soil infested with *Striga hermonthica*.

F. nygamai was described for the first time by Burgess and Trimboli (1986) from Australia. Their initial studies on the occurrence of *F. nygamai* indicate that the fungus occurs in warm temperate subtropical and semiarid regions of several countries. They have isolated the fungus from both symptomless and necrotic roots of sorghum but they have not yet

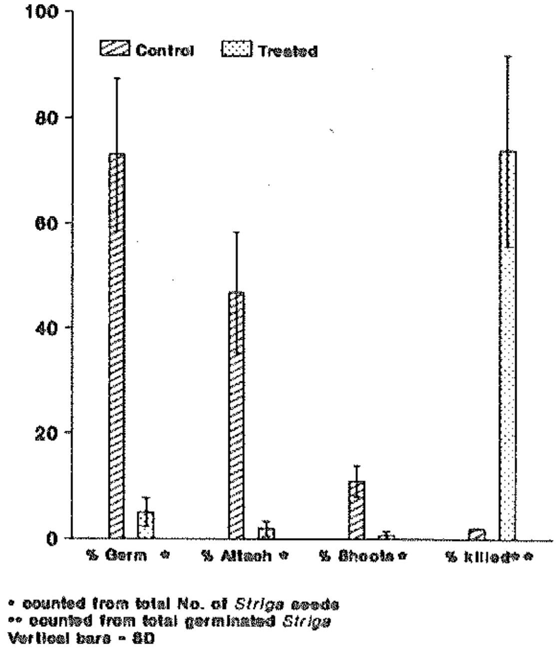

Figure 3. Effect of *Fusarium nygamai* on the underground stages of *Striga hermonthica*.

assessed its ability to cause root rot to this crop or the other plant species from which it has been isolated. Our isolate was found to be specific to *Striga* plants and it did not infect sorghum or any of the other plant species when it was tested for pathogenicity against them. The present data suggest that *F. nygamai* can be highly aggressive towards *S. hermonthica* and it may be considered as a possible agent of biological control potential of this parasitic weed. Since *F. nygamai* was reported to be toxic to ducklings (Onyike *et al.* 1989), the toxicity of this fungus to animals and human beings should be investigated and further studies regarding its performance in the field should also be carried out.

Table 4. Host range of an isolate of *Fusarium nygamai* pathogenic to *Striga hermonthica*.

Plant Species	Disease Reaction[1]
Allium cepa L.	–
Arachis hypogaea L.	–
Brassica oleraeeae var. *botrytis* L.	–
Citrullus vulgaris Schrad.	–
Cucumis sativus L.	–
Helianthus annuus L.	–
Hibiscus esculentus L.	–
Hordeum vulgare L.	–
Gossypium barbadens L. var. *Barakat*	–
Medicago sativa L.	–
Phaseolus vulgaris L.	–
Pisum sativum L.	–
Sesamum indicum L.	–
Sorghum bicolor L. Moench	–
Spinacea oleracea L.	–
Triticum vulgare Vill.	–
Vicia faba L.	–
Zea mays L.	–

[1]Reaction: – = no reaction; + = plant infected.

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