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Evaluation of *Fusarium semitectum* var. *majus* for biological control of *Striga hermonthica*

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Abstract. *Fusarium semitectum* var. *majus* was identified as a soil-borne pathogen of *Striga hermonthica* (Del.) Benth. in the Sudan. Inoculum was produced on sorghum seeds and evaluated in sterilized and unsterilized soil in a pot experiment. Complete control of striga and enhanced host growth were achieved when 10 g of inoculum per kg of soil was incorporated, before planting. In root-chamber trials, the application of *F. semitectum* var. *majus* at a concentration of 4×10^6 spores ml^{-1} reduced germination of striga seeds, attachment of haustoria, and the number of unemerged striga shoots by 83, 93 and 90% respectively. Temperature regimes and the media used interacted to affect fungal growth. Optimal constant and alternating temperatures for the growth of the fungus were 25°C and 20-30°C, respectively. Potato dextrose agar (PDA) was the best medium for growth of the fungus. The fungus did not infect sorghum and its pathogenicity to other crop species was not investigated. Further studies into host range and performance under field conditions should be conducted.

Introduction

Striga spp. (Scrophulariaceae) are hemi-parasitic weeds found mainly in the old-world tropics and subtropics. Of the 31 taxa occurring in Africa, *S. hermonthica* is the most economically important species (Raynal-Roques 1987). A single plant may produce up to half a million dust-like seeds per annum (Andrews 1945) which can remain viable in the soil for more than 15 years. *Striga hermonthica* attacks sorghum, millet and maize, damaging its host before emerging above the ground. A variety of striga control methods including cultural practices, resistant varieties, fertilizers, herbicides, and artificial stimulation of striga seed germination have been investigated. No single method can solve the problem, but a long-term integrated approach is required which could benefit from research into the potential for biocontrol (Parker 1991).

Recently our efforts have concentrated on investigating natural enemies (insects and fungi) of striga as a means of control. In the past 25 years extensive work on biological control of weeds with fungal plant pathogens has led to three approaches: (i) the inoculative method, also called the 'classical strategy' (Templeton 1982); (ii) the augmentative tactic (Phatak *et al.* 1987); and (iii) the inundative method, or the mycoherbicidal approach (Templeton *et al.* 1979; Templeton 1982; Charudattan 1984, 1985). The mycoherbicidal approach consists of applications of massive doses of inoculum to the weed population to create an epidemic rapidly when climatic conditions are favourable. Mycoherbicides are particularly well suited to the control of weeds in annual crops and may give comparable or better control than chemical herbicides. They are highly specific and can control weeds in cropping situations where chemicals would not be sufficiently selective.

Striga is attacked and killed by fungi under natural field conditions, suggesting that soil-borne pathogens may play an important role as natural enemies of the weed. The most common soil-borne pathogens associated with infected striga plants were identified as *Fusarium* spp. (Abbasher and Sauerborn 1992a, b; Kirk 1993; Sauerborn *et al.* 1994; Ciotola *et al.* 1995). Ten *Fusarium* spp. isolated from the Sudan were screened for their potential to reduce *S. hermonthica* emergence through infection of seeds and the early growth stages (before and after attachment to the host). Among them, *F. nygamai* and *F. semitectum* var. *majus* were found to be very effective, reducing the emergence of striga plants by more than 80% when propagated on V-8 medium and incorporated into the soil at a rate of 20 g kg^{-1} of soil before planting (Abbasher and Sauerborn 1992b). Established striga plants were also infected and killed. The sorghum host was not affected by either fungus, and, as a result of striga control, its performance was improved significantly. This work was undertaken to assess: (i) the competitive ability of *F. semitectum* var. *majus* compared to other soil micro-organisms; (ii) the effect of different levels of inoculum on the control of *S. hermonthica*; (iii) the effect of the fungus on the underground stages of striga; and (iv) the thermal and nutritive requirements of the fungus.

Materials and methods

Sorghum and striga seeds were provided by the Department of Botany and Pathology (Agricultural Research Corporation, Wad Medani, Sudan). The seeds were stored at room temperature under dry and dark conditions until used. In all experiments a loam/sand mixture of 2:1 (V/V) was used. The substratum was sterilized for 4 h using steam sterilization apparatus. All pot and root chamber experiments were conducted in a glasshouse at relative humidities that ranged from 25-50% RH and temperatures of between 22°C and 35°C. The day length was approximately 12 h. Supplementary light was provided from HQLR-lamps (1000 W), when required.

Pot experiments

Two pot experiments were performed under glasshouse conditions to study the ability of *F. semitectum* var. *majus* to control striga plants, using sterilized and unsterilized soils, and to evaluate the effect of inoculum dose. In both experiments, five sorghum plants and 100 mg of striga seeds (95% viability) were sown per pot (18 cm diameter). Sorghum grown in uninoculated pots, with and without striga, served as controls. Water was applied as necessary. Two weeks after sowing, sorghum plants were thinned to two plants per pot and fertilized with 200 ml of 2% l^{-1} Superal Wuxal N-P-K (8-8-6). Fungal inoculum was propagated on sterilized sorghum seeds as described by Abbasher and Sauerborn (1992b). To study the effect of *F. semitectum* var. *majus* on the control of striga and its competitive ability with other soil micro-organisms, sterilized and unsterilized soils were used. Inoculum was incorporated into the soil at a rate of 10 g kg^{-1} of soil before planting. To evaluate the effect of inoculum dose on the control of striga seeds and plants, and on the performance of the host plant sorghum, different amounts (0, 2, 5, and 10 g kg^{-1} of soil) of the fungus inoculum were applied. A randomized complete block design with five replicates was used. The experiments lasted for three months and were repeated once. The parameters used to assess the effect of *F. semitectum* var. *majus* on the control of striga and the performance of the host plant sorghum were: striga incidence (weekly counts of emerged striga), sorghum height (measured every two weeks after the first emergence of striga), and dry weight of sorghum shoots, determined after drying at 120°C for 24 h. The data were subjected to analysis of variance and significant differences in the mean values were determined by Fisher's LSD at $P < 0.05$.

Root chamber experiments

Fusarium semitectum var. *majus* was evaluated in root chambers to assess its ability to control the underground stages of striga. Root chambers were used as described by Linke and Vogt (1987) and modified for the application of fungal mycelium and spores, as described by Abbasher and Sauerborn (1992a, b). Ten ml of mycelium and spore suspension (approximately 4×10^6 spores ml^{-1} of deionized water), prepared from 14-day-old fungal cultures, was added to cover the surface of the filter paper. Deionized water served as the control.

The effect of various inoculum concentrations of *F. semitectum* var. *majus* on the underground stages of *S. hermonthica* was evaluated in root chambers. Spore concentrations of 0, 4×10^6 and 8×10^6 spores ml^{-1} of deionized water were used. Otherwise the experiment was similar to the one described above.

The total number of germinated striga seeds, the number of haustoria attached to sorghum roots, the number of unemerged striga shoots, and the number of striga shoots which had been killed by the fungus were counted weekly. Five evaluations were made and the experiments were repeated once. The data were arcsin transformed and analyzed by analysis of variance. Significant differences among means were determined by Fisher's LSD at $P < 0.05$.

Culture of Fusarium semitectum *var.* majus

Fusarium semitectum var. *majus* was assayed for its thermal and nutritive requirements. The fungus was grown on vegetable juice agar (V-8), potato dextrose agar (PDA), or water agar (WA) in 9 cm diameter petri dishes. The inoculum was a 10-mm-diameter disc placed at the centre of the petri dish. The inoculated plates were kept in incubators at different temperature regimes with a photoperiod of 12 h and a light intensity of 6000 lx. The constant temperatures were 10, 15, 20, 25, 30, 35, and 40°C. The alternating temperature regimes were 10-20°C, 15-25°C, 20-30°C, 25-35°C, and 30-40°C (12-12 h). Each treatment included six replicates and was grown for two weeks. Colony size was measured daily. The experiment was repeated once. Data were analyzed as a factorial experiment between the temperature and the media.

Results

Pot experiments

The incidence of striga was strongly reduced when *F. semitectum* var. *majus* was incorporated into the soil, before planting, at a rate of 10 g kg^{-1} of soil. In sterilized and unsterilized soils, emergence of striga was reduced by 93 and 100%, respectively. Sorghum tended to grow better in unsterilized soil, but, the most significant factor enhancing height and dry weight of sorghum shoots in striga-infested pots was treatment with the biocontrol agent (Table 1).

Table 1. *Striga hermonthica* incidence and the performance of sorghum as influenced by *Fusarium semitectum* var. *majus*. Evaluation took place 12 weeks after sowing of sorghum and striga. Data are presented as the mean of one trial with 5 replicates and 2 sorghum plants per pot. S - sterilized soil, US - unsterilized soil. a - soil was infested with 100 mg striga seeds per pot before sowing of sorghum seeds. b - inoculum was propagated on sorghum seeds and incorporated into soil before planting.

Treatment	Soil type	No. of emerged *Striga* pot^{-1}	Sorghum shoot height (cm)	Sorghum shoot dry weight (g)
-Fungus -striga	S	0.0	133.3	26.7
	US	0.0	156.0	27.9
-Fungus +striga[a]	S	21.2	47.6	1.8
	US	13.2	49.9	2.1
+Fungus[b] +striga[a]	S	1.2	154.3	44.8
	US	0.0	165.7	55.8
LSD at P=0.05			20.7	8.4

Table 2. Incidence of *Striga hermonthica* and performance of sorghum as influenced by different amounts of *Fusarium semitectum* var. *majus*. See text and Table 1 for details. Control - sorghum and striga plants without fungal inoculum.

Inoculum (g kg^{-1} soil)	No. of emerged striga per pot	Sorghum shoot height (cm)	Sorghum shoot dry weight (g)
0 (control)	21.6	47.6	1.8
2	3.8	92.0	13.7
5	2.4	123.4	21.7
10	1.0	154.3	44.8
LSD at P = 0.05	-	31.5	7.8

Increasing inoculum application-rate enhanced the ability of the fungus to control striga. Application rates of 2, 5 and 10g kg^{-1} of soil, reduced the incidence of striga by 82, 89 and 95%, respectively, compared with the control (Table 2). Emerged striga plants were attacked and killed. Highly significant increases in sorghum height and shoot dry-mass resulted from the presence of *F. semitectum* var. *majus* in the soil of striga-infested pots.

Root chamber experiments

The fungus reduced the germination of parasite seeds by 46% and was very effective in preventing germinated striga from attaching to the host roots. Compared to the control, 87% of germinated striga failed to attach to the host roots due to the infection. Consequently, the number of striga shoots was reduced by 89% (Fig. 1). *Fusarium semitectum* var. *majus* killed 83% of the germinated striga seeds by attacking their germ tubes, which were observed to change to a dark brown colour and died before attachment to the sorghum roots. Other striga plants were killed following attachment to the host. At a rate of 8×10^6 spores ml^{-1} the fungus reduced the germination of striga by 93%, attachment by 96%, and the unemerged shoots of striga by 94%. The rate of 4×10^6 spores ml^{-1} was almost as effective.

Effect of different temperature regimes and media on the growth of Fusarium semitectum *var.* majus

Environmental factors such as temperature, relative humidity, and nutrient availability limit the development of many fungi as effective biological control agents under natural field conditions. The interaction between temperature and media had a highly significant effect on the growth rate of

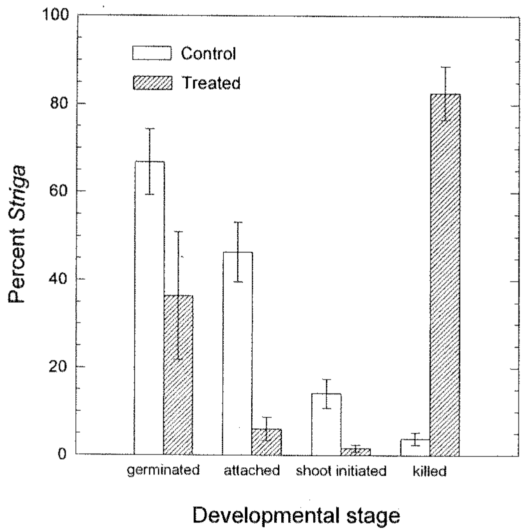

Fig. 1. Effect of *Fusarium semitectum* var. *majus* on the underground development of *Striga hermonthica*. Germination, attachment, and shoot development are presented as a percentage of the number of striga seeds. Killed striga are presented as a percentage of the total that germinated.

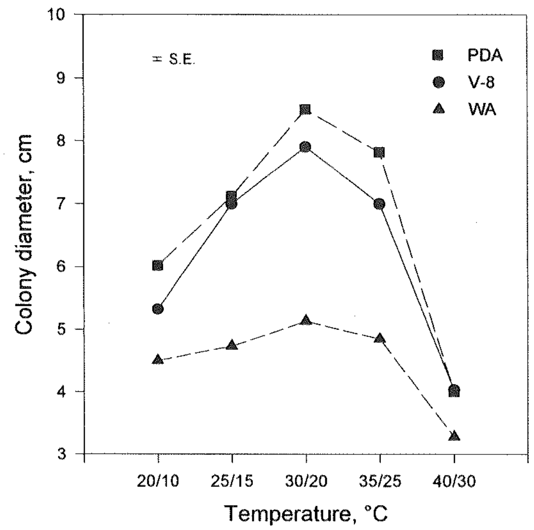

Fig. 3. Effect of alternate temperatures and different media on the growth of *Fusarium semitectum* var. *majus* after seven days. PDA = potato dextrose agar; V-8 = vegetable juice agar; WA = water agar.

F. semitectum var. *majus*. The fungus grew uniformly well on the different media used, but growth was best on PDA (Figs 2 and 3). The optimum temperature for the growth of the fungus was 25°C at a constant temperature and 20-30°C at alternating temperatures (Figs 2 and 3).

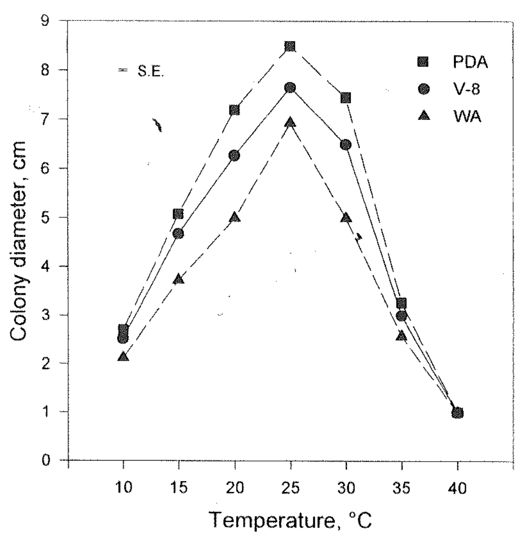

Fig. 2. Effect of constant temperatures and different media on the growth of *Fusarium semitectum* var. *majus* after seven days. PDA = potato dextrose agar; V-8 = vegetable juice agar; WA = water agar.

Discussion

The ten *Fusarium* spp. isolated from the Sudan, are the first soil-borne pathogens to have been evaluated as microbial herbicides against striga (Abbasher and Sauerborn 1992b). The inoculum of the fungus was produced on solid medium (including sorghum seeds) and, when applied before planting, reduced striga infestations at all host growth-stages. Control was achieved in both sterilized and unsterilized soils (Table 1), suggesting that *F. semitectum* var. *majus* is able to grow and compete successfully with other micro-organisms present in the soil.

The use of root chambers allows continuous observations of the early development of root parasites, which is not possible in pot experiments or under natural conditions in the field. In root-chamber experiments the impact of *F. semitectum* var. *majus* on striga germination and attachment was observed. The fungus attacked and killed ungerminated striga seeds, radicles of germinated seeds and striga seedlings attached to the host's roots. These results are similar to those previously reported for *F. nygamai* (Abbasher

Table 3. Effect of different concentrations of *Fusarium semitectum* var. *majus* on the underground stages of *Striga hermonthica* grown in root chambers. Evaluation took place five weeks after sowing of sorghum and striga. The percentages shown are the means of one trial with six replicates. a - 10 ml of each concentration was applied on the surface of the filter paper before addition of striga seeds. b - transformed to arcsin.

Concentrations (spores/ml)[a]	Percent striga germinated	attached	shoots	killed
Control	68.6 (56.2)[b]	43.1 (41.0)	17.6 (24.6)	2.8 (4.3)
4 x 10 ^{6}	10.9 (16.2)	2.9 (8.4)	1.8 (6.4)	77.7 (66.8)
8 x 10 ^{6}	4.5 (11.6)	1.6 (6.0)	1.0 (5.5)	82.7 (73.6)
LSD at P = 0.05	(10.3)	(6.4)	(5.3)	(20.6)

and Sauerborn 1992a, b). Thus the use of *F. semitectum* var. *majus* as a bioherbicide has the potential to reduce striga, both in the year of application, through destruction of germinated and attached striga, and in subsequent years through reduction of the striga seed-bank in the soil.

The saprophytic character of *F. semitectum* var. *majus* led to growth and heavy sporulation on all solid media tested. Therefore the fungus could be applied to the soil in solid (e.g., seed or straw) or granular (e.g., sodium alginate) form. Nutrients available in the substrate extend the period of biological control activity. However, from a practical and economic standpoint, fungi that sporulate in liquid culture are favoured over those requiring other conditions for induction of sporulation. This criterion may prove to be an essential factor for the commercial development of a fungus as a mycoherbicide (Bowers 1982, 1986). This fungal isolate was able to sporulate in all liquid media tested, although the concentration of spores and their type were affected by the media (Thomas 1993). The optimal alternating temperature for the growth of *F. semitectum* var. *majus* was 20-30°C, which coincides with that required for germination and attachment of *S. hermonthica* (Dawoud and Sauerborn 1994). It is clear that the endemic pathogen *F. semitectum* var. *majus* is highly adapted to its target weed, *S. hermonthica*. Moreover, soil incorporation of the fungus reduces its exposure to extremes of moisture and temperature, which otherwise limits the use of foliar bioherbicides in tropical and subtropical areas.

Recently, *F. semitectum* var. *majus* was isolated from *S. asiatica* collected from Madagascar (Kroschel in preparation). Therefore the pathogenicity of this fungus to other *Striga* spp. and its host specificity should be determined before undertaking field trials.

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