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Integrating *Fusarium oxysporum* f. sp. *strigae* into cereal cropping systems in Africa

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Abstract

BACKGROUND: *Striga hermonthica* (Del.) Benth. (witchweed) poses the greatest biological constraint to food production in sub-Saharan Africa (SSA). Control options for *Striga* are currently largely ineffective or unavailable to farmers, and other management possibilities are urgently needed. Biological control obviates some of the problems of several of the other techniques and provides a management option that is durable and environmentally responsive. The efficacy of *S. hermonthica* control using different formulations of three isolates of *Fusarium oxysporum* Schlecht. emend. Synder & Hans f. sp. *strigae* was tested on *Striga*-resistant and *Striga*-susceptible varieties of sorghum and maize under African field conditions for the first time.

RESULTS: Isolates PSM197 and Foxy 2 were effective in witchweed repression, especially when applied as pesta granules. Isolate M12-4A was less effective under the field conditions investigated. Application of the fungi was generally more beneficial in maize than in sorghum for the varieties tested. Application of the biocontrol agent caused significant decreases in the number of flowering *Striga* plants, and hence deposition of seeds with impact of enhancing future crop yield.

CONCLUSIONS: Synergistic effects between the *Striga*-resistant maize line and *Fusarium oxysporum* f. sp *strigae* led to over 90% reduction in *Striga* emergence. These results will further encourage the distribution of the isolates tested or selection of country-specific relatives as viable and environmentally safe biocontrol agents to be used against *Striga*. Pesta was the most effective formulation, while seed coating may be more cost effective.

Keywords: bioherbicide; cereals; formulation; mycoherbicide; *Fusarium oxysporum*; witchweed; *Striga*

1 INTRODUCTION

The parasitic seed plant that poses the greatest economic threat to agriculture worldwide is *Striga hermonthica* (Del.) Benth. (purple witchweed). It negatively impacts the production of cereal crops such as millet [*Pennisetum americanum* (L.) Leeke], sorghum [*Sorghum bicolor* (L.) Moench] maize (*Zea mays* L.), rice (*Oryza sativa* L.) and sugar cane (*Saccharum officinarum* L.), and poses a potential threat to the recently identified susceptibility of wheat (*Triticum aestivum* L.).[1–3] Approximately 50 million ha on the African continent are infested by *Striga* spp., resulting in the loss of more than 10 million t of grain, excluding maize, in 30 countries ranging from Senegal to South Africa.[4,5] Almost all African farmers view *Striga* as the main challenge in agriculture.[4] A diversified array of tools and techniques have been developed by farmers and agronomists to limit damage, but control options for *S. hermonthica* are often ineffective on their own, and need to be supplemented with other management possibilities to produce an integrated package that provides durable control.

Biological control, especially the use of fungal pathogens against *Striga hermonthica*, has gained considerable attention in recent years. Several field surveys in Mali, Niger, Nigeria, Ghana, Sudan and Burkina Faso have led to the conclusion that *Fusarium oxysporum* Schlecht. emend. Snyder & Hans is the most virulent pathogen affecting the development of different witchweed species.[1,6–8] Fungi are preferred to other microorganisms as bioherbicides, given that they are usually host specific, highly aggressive, easy to mass produce and diverse in terms of number of isolates.[9] Different isolates are likely to be adapted to the different host populations and varied environmental conditions that exist across sub-Saharan Africa (SSA). Studies have shown that *F. oxysporum* isolates M12-4A, PSM197 and Foxy 2 are host restricted and only infect plants in the genus *Striga*, and thus constitute the formae speciales *strigae.*[7,10,11] *Fusarium oxysporum* f. sp. *strigae* can saprophytically colonize the root system of the cereal host and subsequently cause infection during all of *Striga* spp. developmental stages.[7,12,13] Laboratory and preliminary field studies found that isolate M12-4A decreased witchweed emergence by 92%, while isolate PSM197 resulted in a reduction of 90%.[9,14]

In order to reduce inoculum requirement (and hence cost) for field inoculations, *F. oxysporum* f. sp. *strigae* has been formulated as pesta granules added to cereal planting holes, or coated directly onto cereal seed using gum arabic.[9,14,15] These methods differ

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in terms of cost and labor as determined by the amount of inoculum required per hectare, the quantity and type of materials needed to produce inoculum and method of on-site application. These considerations alter the way formulated products could be perceived and managed in SSA.

In this study, the agronomic potential of the seed coating and pesta techniques of three different isolates of *F. oxysporum* f. sp. *strigae* were evaluated on *Striga*-resistant and *Striga*-susceptible varieties of sorghum and maize, two cereal crops that are staples of the West African food system. Prototype formulation procedures were used to compare the efficacy of the two delivery methods in the same experiment, something never done thus far. The research was conducted in rain-prone Northern Benin and repeated in drought-prone Eastern Burkina Faso.

2 MATERIALS AND METHODS

2.1 Fungal and plant material

The isolates of *F. oxysporum* f. sp. *strigae* used in this study were M12-4A collected in Mali, PSM197 from Nigeria and Foxy 2 from Ghana.[7,10,16] All *S. hermonthica* seeds used in this experiment were harvested in 2006 at Ina, Benin, from fields where the same crop as that used in the experiments, sorghum or maize, had been grown the previous year to ensure the best possible rate of witchweed germination.[17] *Striga*-susceptible and *Striga*-resistant sorghum varieties Kourboula and sarias-o14 were used. Both originate from Burkina Faso and are early maturing varieties [harvest about 110 days after sowing (DAS)]. The *Striga*-susceptible and *Striga*-resistant maize varieties utilized were 8338-1 and TZL Comp1-SYN-WF2 respectively. Both of these are early maturing.

2.2 Inoculum production

A single-stage liquid fermentation procedure was used for the mass production of chlamydospores of each fungal isolate.[12,18] Maize straw was ground using a Romer electrical blender, and 5 g of ground straw was mixed with 100 mL of distilled water in a 250 mL Erlenmeyer flask to give a 5% (w/v) suspension. The substrate was autoclaved at 121 °C at 15 psi for 15 min, cooled and inoculated with agar plugs (5 mm^2) of five-day-old cultures of the three different *F. oxysporum* f. sp *strigae* isolates grown on potato glucose agar (PGA). Inoculated flasks were incubated at 25 ± 1 °C for 10–15 days on a rotary shaker at 200 rpm (GFL, Istanbul, Turkey). The suspensions were harvested, and chlamydospore concentration was adjusted to 1.8×10^6 mL^{-1} for the seed coating and 1.0×10^6 mL^{-1} for the pesta granules.[19] The concentrations differed between the two formulations in order to diminish the forecast inoculum variation between the two delivery methods, as more chlamydospores are expected in the plots treated with pesta granules.[11,12]

2.3 Formulation procedures

For seed coating, 50 mL of the chlamydospore suspension was added to 20 g of gum arabic in an Erlenmeyer flask and thoroughly mixed. A quantity of 40 mL of this solution was used to coat 400 g of maize seeds, and 10 mL of the solution was used to coat 100 g of sorghum seeds. These rates are similar to those used in previous studies.[12] Coated seeds were allowed to dry overnight on newspaper at room temperature. On the subsequent day, a second coating of inoculum was applied following the same procedure.

The pesta formulation followed an established protocol.[11] For each treatment, 1200 g of durum wheat semolina (Natco brand, UK) was mixed with 225 g of kaolin, 75 g of sucrose and 863 mL of fungal inoculum adjusted to 10^6 chlamydospores mL^{-1} of liquid substrate. The resulting dough was pressed, dried for 72 h and then milled into granules with a household grinder. For the controls without *F. oxysporum* f. sp. *strigae*, 863 mL of distilled water was added to the mix instead of fungal inoculum.

The total amount of coated inoculum applied per seed hole at sowing was dependent on the fact that three maize seeds or five sorghum seeds were planted in each hole. These numbers are consistent with cultural practices of local farmers and limit the variation in inoculum quantity placed in each pocket owing to the seed size disparity between maize and sorghum. By comparison, 2 g of pesta granules was added for each seed hole for both crops.

The final concentration of chlamydospores of the different formulations was determined by placing 30 seeds in 10 mL of distilled water or 0.5 g of pesta granules in 10 mL of water and shaking on a vortex mixer for 1 min. Two counts were performed using a haemocytometer to determine the number of chlamydospores per seed or g of pesta. In order to ensure that seeds coated with gum arabic and the biocontrol agent could germinate freely, seeds were placed on moist filter paper in the dark at 25 °C for 5 days, and germination rates were determined and compared with untreated seed. Finally, coated seeds and pesta granules were plated on PGA at 25 °C for 5 days to confirm the viability of the fungal inoculum.

2.4 Experimental design

Field studies were arranged as randomized complete block designs, with four replicate blocks of 107.25 m by 4.5 m each per crop. Each plot consisted of four rows of 4.5 m with a plant spacing of 0.5 m and a distance between plots of 0.75 m to respect customary farming procedures in maize and sorghum cropping systems. Treatments included three different isolates of *F. oxysporum* f. sp. *strigae*, M12-4A, PMS197 and FOXY 2, in two formulations, pesta granules and seed coating, plus two formulation controls without fungus, plus two controls with and without sowing of *S. hermonthica* seed. All treatments, except the control without *S. hermonthica* seeds, were infested with *S. hermonthica*. Each of the above ten treatments was applied to both the resistant and susceptible varieties, yielding a total of 20 treatments for each crop (sorghum or maize). The trials were conducted in the Beninese village of Ina (82 km north of Parakou) and repeated in the Burkinabe community of Fada during the 2007 cropping season.

2.5 Field manipulations

Three maize and five sorghum seeds were added per planting hole of 1.5 cm depth, and plants were thinned to one plant per hole, ten plants per row following emergence. *Striga* infestation was achieved through the use of a calibrated scoop containing a *Striga*–sand mix that delivered 3000 seeds per planting hole.[20] Pesta granules were added to planting holes at sowing. Fertilizer was applied by hand as spoonfuls per seeding hole, 30 DAS, to provide a field dressing of 80 kg ha^{-1} of 14–23–14 ($N-P_2O_5-K_2O$). This rate was selected on the basis that local traditional farming techniques routinely used this amount, which was available as a consequence of leftover applications to cotton crops.

In Benin, data were recorded at 25, 53 and 98 DAS for maize and in addition at 109 DAS for sorghum. The height of cereal plants at the highest vegetative point was recorded, and the numbers of emerged, diseased and flowering *Striga* shoots per plant were assessed. Parasitic plants further than 38 cm from the exterior of the sampling rows were not counted. Diseased *Striga* plants were defined as either showing symptoms of vascular wilt or being completely dead (wilted and blackened). Maize cobs (ears) and sorghum panicles were harvested 98 and 109 DAS respectively. The above-ground biomass of *S. hermonthica* was collected after the cereals were harvested. All plant material was dried in ovens at 80 ± 1 °C for 72 h and then weighed.

In Burkina Faso, evaluations were performed 30, 60 and 90 DAS. The same field data were collected as described for Benin, with the exception of counts of flowering and diseased *Striga* plants. Maize cobs and stems were harvested 100 DAS, whereas the sorghum panicles and stems were harvested 121 DAS. *Striga hermonthica* plants were also sampled and sun dried for 72 h.

2.6 Statistical analysis

The data were analyzed using SAS software version 9.1.[21] Variances of experiments were not homogeneous, thus the sites and cultivars were analysed separately. Tests for normality using analyses of residuals with PROC UNIVARIATE were performed using the Kolmogorov–Smirnov and Shapiro–Wilk tests.[21] Non-normally distributed data were transformed with the square root transformation.[22] Two-way analyses of variance (ANOVA) and correlation analyses were performed on the data. Means were compared using least significant difference (LSD) with $\alpha = 0.05$.

3 RESULTS

3.1 Inoculum concentrations

The amount of inoculum used in maize was similar for both varieties (Table 1). For sorghum, twice the number of chlamydospores were coated onto the susceptible as opposed to the resistant variety seeds. The larger seeds of the susceptible variety provided a greater surface area for coating with gum arabic and fungal inoculum. Formulation of inoculum into pesta granules followed by an application rate of 2 g per planting hole delivered a considerably greater amount of inoculum than seed coating (85–244 times more chlamydospores). The quantities of fungal propagules counted in the formulations were larger than those used in previous studies.[9,12]

3.2 *Striga* dry weight and emergence in maize

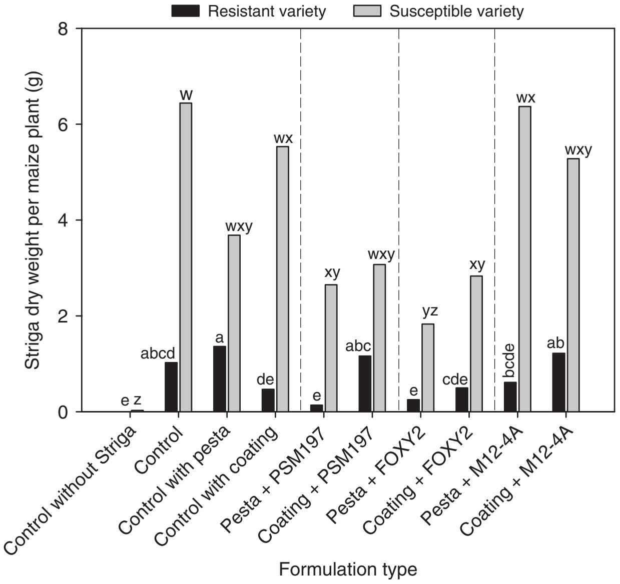

Figure 1. Effect of *Fusarium oxysporum* f. sp. *strigae* isolates and bioherbicide formulation on *Striga hermonthica* above-ground dry weight in resistant and tolerant maize in Benin. Means sharing the same letter in either the black or gray bars are not significantly different at $P < 0.05$ according to LSD.

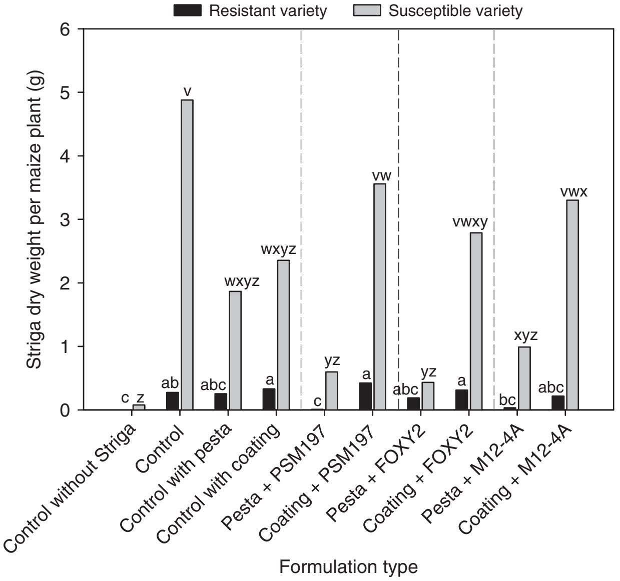

Figure 2. Effect of *Fusarium oxysporum* f. sp. *strigae* isolates and bioherbicide formulation on *Striga hermonthica* above-ground dry weight in resistant and tolerant maize in Burkina Faso. Means sharing the same letter in either the black or gray bars are not significantly different at $P < 0.05$ according to LSD.

Table 1. Number of *Fusarium oxysporum* f. sp. *strigae* chlamydospores on coated seeds and in pesta granules

Coated seeds	Chlamydospores per seed hole	Published unitary rates (chlamydospores)
Resistant maize	4.4×10^6	Unknown
Susceptible maize	4.6×10^6	
Resistant sorghum	1.6×10^6	1.6×10^{3a} to 2.0×10^{5b}
Susceptible sorghum	4.0×10^6	
Pesta	3.9×10^8	1.0×10^{6c} to 1.4×10^{7d}

[a] Reference 12.
[b] Reference 9.
[c] Reference 15.
[d] Reference 11.

Striga plants were found to emerge in uninfested control plots, especially in Burkina Faso, suggesting that background witchweed populations existed at the trial sites. An analysis of variance on *Striga* dry weight and emergence in the maize fields revealed strong treatment effects in Benin and Burkina Faso ($P < 0.05$). Furthermore, weed dry weights and emergence were strongly correlated with coefficients of 0.71 and 0.82 in Benin and Burkina Faso respectively ($P < 0.05$). The resistant maize variety provided a significant level of witchweed control in both countries (Figs 1 and 2). Both PSM197 and Foxy 2, and to a lesser extent M12-4A, decreased witchweed dry weight and emergence. For all isolates tested, application of fungal inoculum via pesta granules was the most efficient control option. It was interesting to note that M12-4A provided greater control when applied as pesta in Burkina Faso compared with Benin (Figs 1 and 2). For the resistant variety, seed coating with Foxy 2 suppressed *Striga* in Benin but not in Burkina Faso or for other isolates when compared with the control. For the susceptible variety, seed coating with both Foxy 2 and PSM197 provided significant control in Benin and to a lesser extent in Burkina Faso, while M12-4A was ineffective.

For all treatments applied to susceptible maize, with the exception of fungus applied as pesta, more parasitic plants emerged at the first sampling date (53 DAS) than at the second (96 DAS) (data not presented). For pesta applications to the susceptible variety of maize, and all treatments applied to the resistant variety, the number of witchweed plants was higher at 96 DAS than at 53, demonstrating that *Striga* developmental rate was reduced. Similar results were not observed in Burkina Faso, presumably because drought conditions, 60 DAS, limited weed development (data not presented). Subsequent rains permitted *Striga* emergence between 60 and 90 DAS, overriding the variety and fungal impacts on *Striga* development.

3.3 *Striga*: diseased and flowering for maize in Benin

There were significantly more diseased weed plants 96 DAS observed on *Striga*-susceptible maize when compared with the resistant variety (data not presented). The presence of diseased *Striga* plants suggests that plant kill was not achieved prior to weed emergence, so the efficacy of a biocontrol treatment cannot be directly linked to these data. Occasionally in control treatments, without the addition of the biocontrol agent, diseased *Striga* plants were present, suggesting that *F. oxysporum* or other similar pathogens occurred at the field site.

For the susceptible maize variety the application of PSM197 and Foxy 2, irrespective of the formulation, significantly decreased the number of flowering *Striga* plants (Fig. 3), while M12-4A produced less pronounced effects on *Striga* flowering. For the resistant variety, significant reductions in the number of flowering *Striga* plants were only found when PSM197 and Foxy 2 were applied as pesta granules.

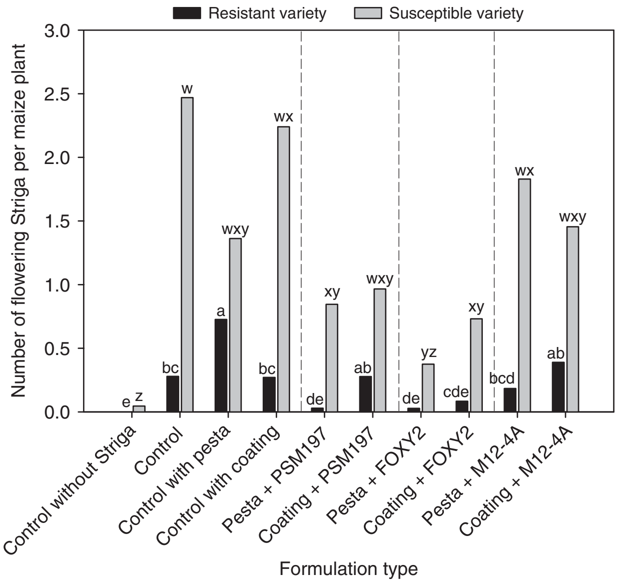

Figure 3. Effect of *Fusarium oxysporum* f. sp. *strigae* isolates and bioherbicide formulation on the number of flowering *Striga hermonthica* in resistant and tolerant maize 96 days after sowing in Benin. Means sharing the same letter in either the black or gray bars are not significantly different at $P < 0.05$ according to LSD.

3.4 Maize heights and final yields

Maize height at the end of the Beninese growing season was significantly influenced by the applied treatments ($P < 0.05$) (data not shown). Biocontrol agents applied as pesta granules promoted crop growth, especially for the susceptible variety, while seed coating treatments were less effective. In Burkina Faso, treatment effects were less, likely a consequence of the drought conditions. The susceptible maize variety used in Burkina Faso was possibly more drought tolerant than the *Striga*-resistant variety on the basis of increased plant height. The correlation between *Striga* emergence and cob yield was moderate ($r = -0.57$ with $P < 0.01$) in Benin but non-existent in Burkina Faso ($r = 0.004$ with $P < 0.96$).

In terms of cob yield, a strong treatment effect was noted in Benin, mainly owing to the increased yield produced by the resistant variety compared with the susceptible variety ($P < 0.05$) (Fig. 4). None of the formulations tested significantly increased the yield of the susceptible variety when compared with the control with *Striga*. In Burkina Faso, no significant treatment main effects were recorded ($P < 0.51$); the unavailability of water probably restricted cereal yields, especially of the resistant cultivar. However, yield of the susceptible variety was significantly increased with PSM197 pesta, and M12-4A pesta also substantially increased crop yield (Fig. 5).

3.5 *Striga* dry weights and emergence in sorghum

The different formulations had strong impacts on witchweed above-ground biomass and emergence in Benin and Burkina Faso ($P < 0.05$) (Figs 6 and 7). However, in contrast to results for maize, only moderate correlation coefficients were observed between

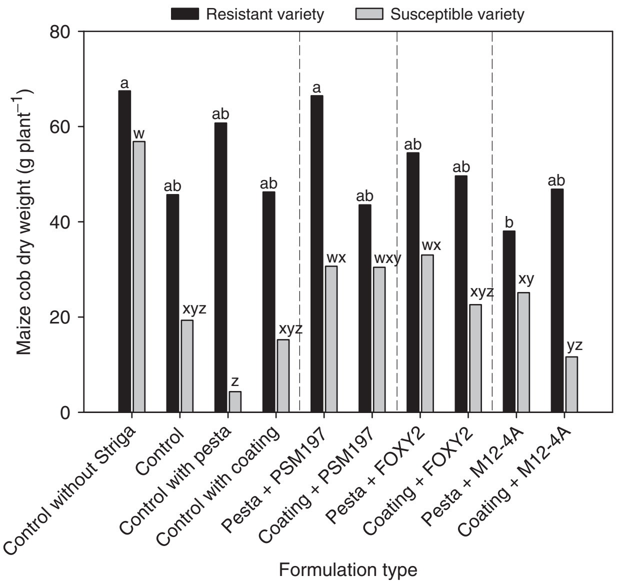

Figure 4. Effect of *Fusarium oxysporum* f. sp. *strigae* isolates and bioherbicide formulation on resistant and tolerant maize cob dry yields in Benin. Means sharing the same letter in either the black or gray bars are not significantly different at $P < 0.05$ according to LSD.

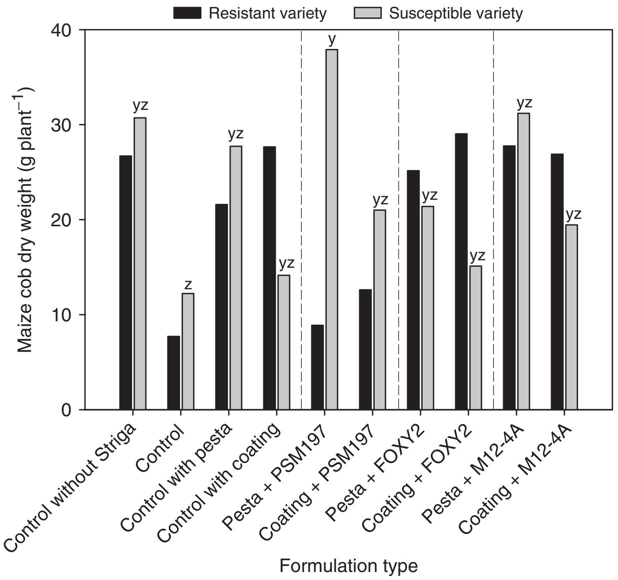

Figure 5. Effect of *Fusarium oxysporum* f. sp. *strigae* isolates and bioherbicide formulation on resistant and tolerant maize cob dry yields in Burkina Faso. Means sharing the same letter in either the black or gray bars are not significantly different at $P < 0.05$ according to LSD. There were no significant differences in yield data among treatments of the resistant cultivar.

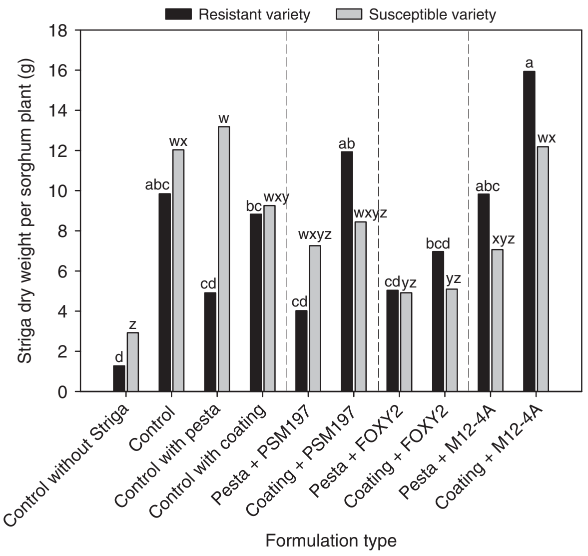

Figure 6. Effect of *Fusarium oxysporum* f. sp. *strigae* isolates and bioherbicide formulation on *Striga hermonthica* above-ground dry weight in resistant and tolerant sorghum in Benin. Means sharing the same letter in either the black or gray bars are not significantly different at $P < 0.05$ according to LSD.

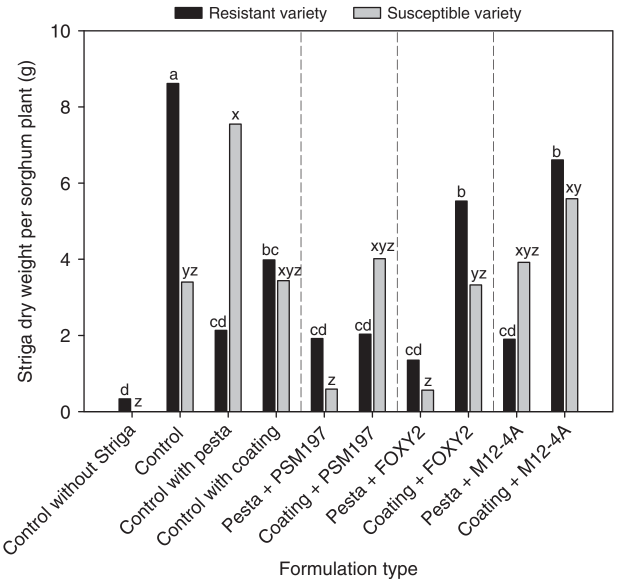

Figure 7. Effect of *Fusarium oxysporum* f. sp. *strigae* isolates and bioherbicide formulation on *Striga hermonthica* above-ground dry weight in resistant and tolerant sorghum in Burkina Faso. Means sharing the same letter in either the black or gray bars are not significantly different at $P < 0.05$ according to LSD.

these two measurements for sorghum in Benin ($r = 0.50, P < 0.01$) and Burkina Faso ($r = 0.47$, $P < 0.01$). Reduced *Striga* dry weight with the application of biocontrol formulations was more evident in the trial in Burkina Faso than in Benin. In Burkina Faso, both PSM197 and Foxy 2 pesta treatments significantly reduced *Striga* in the resistant cultivar, while Foxy 2 significantly reduced *Striga* in the susceptible variety.

A multivariate analysis of variance performed on witchweed emergence over time revealed that there were no interactions between time and treatment ($P < 0.13$ in Benin and $P < 0.55$ in Burkina Faso), indicating that neither the fungus nor the resistant variety were able to delay *Striga* development (data not shown).

3.6 *Striga*: diseased and flowering for sorghum in Benin

At 109 DAS, the ratio of diseased to emerged *Striga* plants and the number of flowering plants in sorghum fields varied significantly with treatments applied ($P < 0.05$) (data not presented). The ratio of diseased to emerged *Striga* plants was higher when the fungus was coated on seeds. As reported for maize, natural infections of witchweed with characteristic wilt symptoms in sorghum control treatments without *Striga* infestation were probably due to naturally occurring pathogen populations.

The use of fungi, particularly PSM197 and Foxy 2, tended to reduce the overall number of flowering *Striga*, with a significant reduction in *Striga* flowering in both the susceptible and the resistant varieties (Fig. 8). Across all isolate applications, pesta granules appeared to be the most effective.

3.7 Sorghum heights and final yields

In terms of crop heights, strong treatment main effects ($P < 0.05$) were observed in both countries (data not shown). These effects are explained by the growth habit of the two varieties being considerably different. The resistant variety was in fact much smaller than the susceptible variety across all treatments. Hence, height of cereals is an unreliable indicator of the effectiveness of the breeding effort to control *Striga*. Benefits of applications of fungi were more evident in Burkina Faso compared with Benin, where effects on sorghum height were only rarely significant. This could be explained by the increased impact of *Striga* on drought-stressed sorghum plants in Burkina Faso. In this country, both pesta and seed coating technologies enhanced crop growth, especially when PSM197 or Foxy 2 was combined with the susceptible variety.

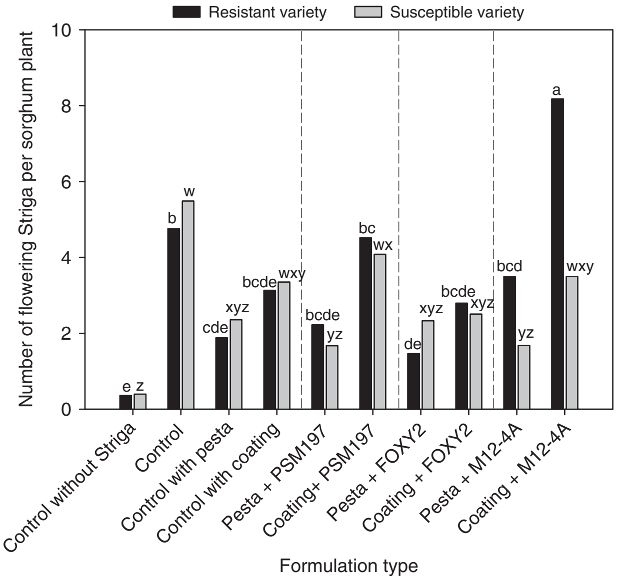

Figure 8. Effect of *Fusarium oxysporum* f. sp. *strigae* isolates and bioherbicide formulation on the number of flowering *Striga hermonthica* in resistant and tolerant sorghum 109 days after sowing in Benin. Means sharing the same letter in either the black or gray bars are not significantly different at $P < 0.05$ according to LSD.

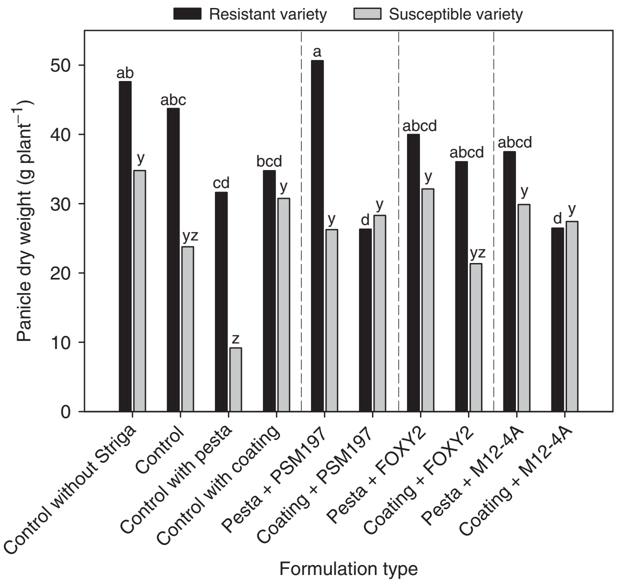

Figure 9. Effect of *Fusarium oxysporum* f. sp. *strigae* isolates and bioherbicide formulation on resistant and tolerant sorghum panicle dry yields in Benin. Means sharing the same letter in either the black or gray bars are not significantly different at $P < 0.05$ according to LSD.

Although treatments had statistical impacts on sorghum yield in Benin ($P < 0.05$), parasitic plant density did not seem to affect yield loss. This is demonstrated by a non-existent correlation between *Striga* emergence and panicle dry weight. The coefficient of correlation in Benin was −0.09 ($P < 0.01$), while in Burkina Faso it was −0.06 ($P < 0.01$). In Benin, controls with and without *Striga* were not significantly different, illustrating that the sorghum crop did not suffer extensively from the parasite (Fig. 9). Some formulations increased the yield when compared with their controls, but never to substantial levels. In Burkina Faso, yields were much reduced when compared with those in Benin. However, for the most part there were no significant effects of treatments on yield of the resistant variety, whereas both PSM197 and coated Foxy 2 significantly increased the yield of the susceptible variety as compared with the *Striga*-infested control ($P < 0.05$) (Fig. 10). It is possible that drought conditions partially masked any impact of *Striga* infestation and hence differing levels of control conferred by the applied treatments.

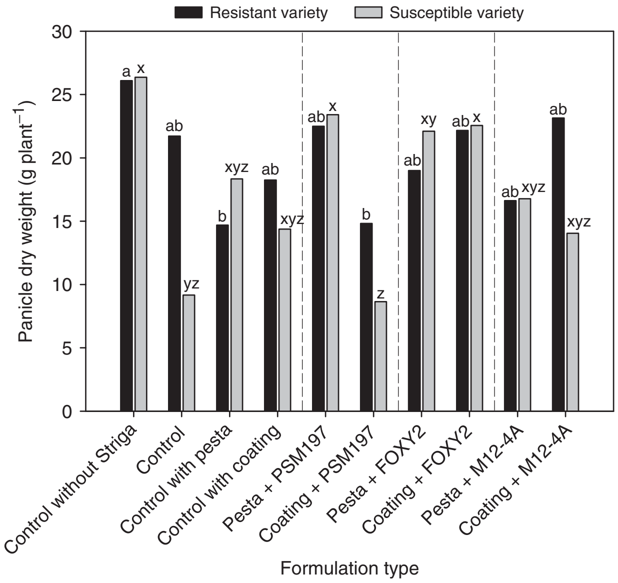

Figure 10. Effect of *Fusarium oxysporum* f. sp. *strigae* isolates and bioherbicide formulation on resistant and tolerant sorghum panicle dry yields in Burkina Faso. Means sharing the same letter in either the black or gray bars are not significantly different at $P < 0.05$ according to LSD.

4 DISCUSSION

4.1 Data comparison

In a pot trial, a 92% reduction in *Striga* emergence in susceptible sorghum was achieved.[9] This was obtained when10^7 chlamydospores of M12-4A were soil applied as a dry powder formulation (ground straw and fungal propagules) in addition to 10 g of ground sorghum straw amendment. A complete reduction in witchweed above-ground biomass was recorded when using the seed coating technology with 2×10^5 spores per sorghum seed. In the present study, M12-4A applied as a seed coating only suppressed emergence by a maximum of 23% in maize and 8% in sorghum compared with the susceptible control, in spite of increased inoculum levels in the formulation. However, when applied as pesta granules, M12-4A reduced *Striga* biomass by 74% in the Benin sorghum and in the Burkina Faso maize. In other formulation/crop/country instances, M12-4A did not demonstrate high virulence except when used with the resistant maize variety in Burkina Faso. In this case, it led to a 93% decrease in parasitic plant biomass when compared with the resistant variety control.

Foxy 2 applied as a seed coating in sorghum has previously been shown to reduce *Striga* emergence by 70% in a pot experiment.[12] In the present study, the same fungus used with the susceptible sorghum crop achieved 39% reduction in *Striga* biomass in Benin but did not significantly affect emergence in Burkina Faso. However, even if the *Striga* control in the latter case was not significant, the seed coating technology contributed to an important increase in sorghum crop height and yield (Fig. 10). Moreover, Foxy 2 achieved 88% reduction in *Striga* biomass in Benin maize when used with the resistant variety compared with its control. The fact that earlier experiments were established in pots might have influenced *Striga* suppression.[12] Since water provides a means of spore dispersal in the soil, a regime of artificial watering in pots could have increased the rate of *Fusarium* soil colonization as opposed to drier field conditions.[23] Moreover, both studies used more fertilizer than was used in the present work. Consequently, a synergistic effect possibly occurred between the biocontrol agent and the fertilizer, leading to more extensive *Striga* repression in their pot trials.

More than 75% reduction in witchweed emergence was reported when using Foxy 2 formulated as pesta granules in sorghum.[11] Similar rates of control occurred when 40 g of colonized wheat grains per pot were applied. A delay in *Striga* emergence in sorghum was previously reported, while this aspect was only clearly observed in maize in this study.[11] Increases in sorghum above-ground biomass reached 100% in that pot study, a rate similar to that observed with PSM197 and Foxy 2 in Burkina Faso in this study (Fig. 10).

Foxy 2 and PSM197, when formulated as pesta granules, brought similar levels of *Striga* control in a field study.[15] On average, both fungi decreased witchweed emergence by 75% in maize and by 55% in sorghum. In the present study, the same pesta formulation of PSM197 led to reductions of 89 and 69% in *Striga* biomass within the maize crops in Benin and Burkina Faso respectively. For Foxy 2, these numbers were 93 and 87% respectively. In sorghum, PSM197 inhibited *Striga* biomass by 60 and 76% in the two countries, while Foxy 2 achieved 56 and 76%. Thus, it appears that the two isolates behaved similarly with sorghum but had diverging virulence in maize, in contrast to previous observations.[15]

4.2 Synergy between control methods

Approximately 96% control was obtained in field studies with the synergy of a resistant variety and either Foxy 2 or PSM197 applied as pesta granules in maize.[15] In sorghum, the rates they observed were 98 and 77% for both fungi respectively.[15] In the present experiment, a combination of a resistant variety and *F. oxysporum* contributed to 97–100% *Striga* reduction when compared with the susceptible variety control in maize. In sorghum, this rate decreased to 41% when PSM197 and the resistant variety were used in Burkina Faso, but averaged 70% otherwise for either this strain or Foxy 2. A combination of seed coating, fungus and the resistant variety resulted in 84–96% *Striga* repression in maize, but did not control more than 58% of the parasitic weed in sorghum.

Even though the resistant maize variety provided excellent control of *Striga*, the combination of pesta granules with fungi should still be encouraged for agronomic reasons to limit the production and deposition of witchweed seed. In plots where this variety was sown and fungus was added as pesta granules or in a few instances as a seed coating, parasitic plants were nearly absent (Figs 1 and 2). Consequently, it could be appropriate to encourage farmers to employ the two technologies together to limit *Striga*'s eventual resistance to either control method in the long term, thus providing durable resistance. A cost efficiency study is required to assess the benefit of increasing the number of control measures implemented under field conditions for subsistence farmers. This is particularly true for comparing seed coating inoculum onto resistant seed and production and application of pesta.

As previously observed, the combination of a resistant variety plus fungus decreased the number of flowering witchweed plants, especially in maize (Figs 3 and 8).[15] The best outcomes were obtained when PSM197 was applied as pesta granules and when Foxy 2 was used in either pesta or seed coating formulations.

The present data demonstrate that the resistant sorghum variety used in this experiment did not lead to expected results. Hence, this mechanism did not reduce the degree of witchweed seed production. Under the scenario where *Striga*-resistant varieties are used in combination with *F. oxysporum* as a biocontrol agent, the basis of the variety's resistance or tolerance should be chosen to favor the use of the fungus. For instance, a sorghum variety that is resistant to *Striga* because of the lack of sorgoleone in its root exudates should be encouraged when using *F. oxysporum*, as this compound possesses antifungal properties.[24] Conversely, a *Striga*-resistant variety, on account of the absence of that strigolactone from the roots, should be discouraged, as this compound is a fungal stimulant shown to improve branching of arbuscular mycorrhizal fungi associated with the cereal. Hence, similar beneficial effects possibly stimulate the crop root colonization by *F. oxysporum*.[25]

4.3 Comparison of pesta granules and seed coating

The greater amount of inoculum in the pesta formulation may be among the main reasons explaining the superior performance of this delivery method when compared with seed coating. Moreover, the granules are applied in the field such that inoculum covers the surface of a greater volume than inoculum applied as a seed coating. Hence, it is likely that *Fusarium* is better able to colonize the planting hole more rapidly and infect *Striga* seeds more extensively when applied as a granulated powder. This colonization might be further encouraged by the sucrose and gluten proteins found in the pesta formulation, providing more nutritive components compared with the arabin carbohydrate gum arabic.[11,12]

It appears that the seed coating technology was more effective in Benin than in Burkina Faso (Figs 1 and 2). This could be explained by the fact that this type of fungal application relies on heavy rainfalls for fungal growth and migration within the soil system. Spore dispersal and fungal colonization in soil only occurs when percolation rates are high. This is particularly true for applications as seed coatings, as spores have a highly hydrophobic nature, making them adhere to gum arabic.[23] Since rainfalls were more frequent in Benin than in Burkina Faso in the season of the experiment, the biocontrol agent most likely colonized the soil more extensively, leading to the observed increases in *Striga* control. Fungus applied in pesta granules is not embedded in a sticky matrix and is dispersed in a greater soil volume. Hence, soil dispersion is expected to be readily achieved with this delivery method, without reliance on rainfall, explaining why it achieved similar results in both countries.

Since the seed-applied biocontrol agent is dependent on the crop host root network to develop, it is possible that the improper sorghum germination observed in the present experiment restrained the fungus from colonizing the soil system.[26] Germination tests performed before sowing demonstrated that coated seeds of both crops were as viable as non-coated seeds, but the seed treatment technology was generally less effective in sorghum than in maize. The greater *Striga* repression in maize might be related to the composition of sorghum root exudates. Sorghum leachates impaired M12-4A development by restraining the elongation of chlamydospore germ tubes.[9] The exact chemical compound that caused inhibition was not identified, but sorgoleones or apigeninidin might play an important role in the process. The use of *F. oxysporum* in a sorghum cropping system should be closely linked with the type of variety used. Further research should address a comprehensive list of varieties that are compatible with the biocontrol agent by identifying those that do not excrete compounds that are inhibitory to fungal growth.

4.4 Lack of treatment main effects in yield analyses

Field experiments with *Striga* frequently have a great deal of variability owing, in part, to unequal *Striga* infections.[27] Analysis of the data from Burkina Faso revealed no strong treatment effects on yield from varied formulation. For maize, the susceptible and resistant variety controls, with and without *Striga*, were not significantly different from one another (Fig. 5). Since inoculation of planting holes with witchweed seeds was performed in a relatively localized manner, it is possible that the crops were parasitized at only specific points of their entire vascular system. Water and nutrient uptake from other sections of the root system may have compensated for this and provided sufficient uptake to maintain normal plant growth and yield formation.

Main treatment effects of the data from Benin on different formulations were observed for crop yield (Figs 6 and 9). However, the addition of *F. oxysporum* f. sp. *strigae* did not consistently increase maize cob or sorghum panicle yield over the controls as previously reported.[15] In the present case, the lack of effect could be related to the frequent rainfalls experienced in most of the Beninese growing season, providing crops with sufficient water to reach a state of resilience towards weed parasitism. Moreover, the rate of *Striga* inoculation in the present study was less than levels used in previous studies, which potentially led to insufficient infestation and crop damage for control treatments.[9,15] A broadcast inoculation of *Striga* at a rate of 10^5 seeds per cereal plant could be a solution to bypass the lack of treatment main effects in yield analysis. However, such a method should be performed with extreme care to avoid drift of the *Striga*–sand mixture outside the field.

4.5 Opportunities to integrate *Fusarium oxysporum* f. sp. *strigae* with other technologies

Seed-coated imidazolinone-resistant maize has been successfully used in Kenya to combat *Striga*, but rain leaches the herbicide away from the root zone.[28] It is speculated that imidazolinone-resistant *F. oxysporum* f. sp. *strigae* mutants could be selected and co-coated with a low dose of imidazolinone herbicides on ALS-resistant maize germplasm to extend the duration of protection from *Striga*. Fungicide-resistant mutants of *F. oxysporum* f. sp. *strigae* could protect against the farmers' fungicide seed treatments, or *F. oxysporum* f. sp. *strigae* could be coapplied with *F. oxysporum* Fo47 for control of pathogenic seed fungi.

Amino acids have been suggested to enhance weed control.[29] In laboratory studies, leucine and threonine inhibited *Striga* germination.[30] The use of such compounds alongside the application of *F. oxysporum* f. sp. *strigae* could enhance *Striga* control. Mutants of PSM197 or Foxy 2 overexcreting leucine and threonine could improve the virulence these isolates currently show on *S. hermonthica*.

5 CONCLUSION

Striga-resistant maize variety TZL Comp1-SYN-WF2 effectively suppressed witchweed. Conversely, sorghum variety sarias-o14 did not achieve significant suppression of witchweed, although it showed elements of tolerance to the effect of parasitism. Application of the fungal biocontrol agent was effective in maize, particularly when inoculum was applied in the form of pesta granules. However, the delivery of the biocontrol agent via seed coating frequently provided satisfactory control and offered the advantage of requiring the production of less inoculum. Hence, the seed coating technology could be considered in regions where the levels of infestation are low or moderate.

The reduced performance of M12-4A in comparison with PSM197 or Foxy 2 in this study may be due to physiological specialization and geography. Physiological specialization is known to occur in *Striga*, as some strains cross-infect host plants while others do not.[31] *Striga hermonthica* is an obligate outcrosser, and studies have shown that geographic distance plays a major role in genetic differentiation among *S. hermonthica* populations. M12-4A was collected in Mali, while PSM197 and Foxy 2 were collected from Nigeria and Ghana respectively. PSM139 and Foxy 2 share the same vegetative compatibility group (VCG), while M12-4A is in a different VCG (Venne *et al*., unpublished).

These results suggest that the adoption of *F. oxysporum* f. sp. *strigae* as a component of integrated *Striga* management should be encouraged for maize. Under conditions of heavy witchweed infestations, the combined use of a resistant variety and fungus applied in pesta granules or seed coating could be recommended. In sorghum, further research is required to address what variety-specific characteristics are needed to achieve improved synergy with the application of the biocontrol agent.

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REFERENCES

1 Elzein A and Kroschel JK, *Fusarium oxysporum* Foxy 2 shows potential to control both *Striga hermonthica* and *S. asiatica*. *Weed Res* **44**:433–438 (2004).
2 Marley PS, Aba DA, Shebayan JAY, Musa R and Sanni A, Integrated management of *Striga hermonthica* in sorghum using a mycoherbicide and host plant resistance in the Nigerian Sudano-Sahelian savanna. *Weed Res* **44**:157–162 (2004).
3 Vasey RA, Scholes JD and Press MC, Wheat (*Triticum aestivum*) is susceptible to the parasitic angiosperm *Striga hermonthica*, a major cereal pathogen in Africa. *Phytopathology* **95**:1294–1300 (2005).
4 Ejeta G, The *Striga* scourge in Africa: a growing pandemic, in *Integrating New Technologies for Striga Control: Towards Ending the Witch-hunt*, ed. by Ejeta G and Gressel J. World Scientific, Singapore, pp. 3–16 (2007).
5 Gressel J, Hanafi A, Head G, Marasas W, Obilana AB, Ochanda J, *et al*, Major heretofore intractable biotic constraints to African food security that may be amenable to novel biotechnological solutions. *Crop Prot* **23**:661–689 (2004).
6 Abbasher AA, Hess DD and Sauerborn J, Fungal pathogens for biocontrol of *Striga hermonthica* on sorghum and pearl millet in West Africa. *African Crop Sci J* **6**:179–188 (1998).
7 Ciotola M, Watson AK and Hallett SG, Discovery of an isolate of *Fusarium oxysporum* with potential to control *Striga hermonthica* in Africa. *Weed Res* **35**:303–309 (1995).

8 Beed FD, Hallett SG, Venne J and Watson AK, Biocontrol using *Fusarium oxysporum*; a critical component of integrated *Striga* management, in *Integrating New Technologies for Striga Control: Towards Ending the Witch-hunt*, ed. by Ejeta G and Gressel J. World Scientific, Singapore, pp. 283–300 (2007).

9 Ciotola M, DiTommaso A and Watson AK, Chlamydospore production, inoculation methods and pathogenicity of *Fusarium oxysporum* M12-4A, a biocontrol for *Striga hermonthica*. *Biocontrol Sci Technol* **10**:129–145 (2000).

10 Marley PS, Kroschel J and Elzein A, Host specificity of *Fusarium oxysporum* Schlecht (isolate PSM 197), a potential mycoherbicide for controlling *Striga* spp. in West Africa. *Weed Res* **45**:407–412 (2005).

11 Elzein A and Kroschel JK, Host range studies of *Fusarium oxysporum* Foxy 2: an evidence for a new forma specialis and its implications for *Striga* control. *J Plant Dis Prot* **20**:875–887 (2006).

12 Elzein A, Kroschel J and Leth V, Seed treatment technology: an attractive delivery system for controlling root parasitic weed *Striga* with mycoherbicide. *Biocontrol Sci Technol* **16**:3–26 (2006).

13 Abbasher AA and Sauerborn J, *Fusarium nugamai* a potential bioherbicide for *Striga hermonthica* control in sorghum. *Biocontrol* **2**:291–296 (1992).

14 Marley PS and Shebayan JAY, Field assessment of *Fusarium oxysporum* based mycoherbicide for control of *Striga hermonthica* in Nigeria. *Biocontrol* **50**:389–399 (2005).

15 Schaub B, Marley P, Elzein A and Kroschel J, Field evaluation of an integrated *Striga hermonthica* management in sub-Saharan Africa: synergy between *Striga*-mycoherbicides (biocontrol) and sorghum and maize resistant varieties. *J Plant Dis Prot* **20**:691–699 (2006).

16 Abbasher AA, Kroschel J and Sauerborn J, Microorganisms of *Striga hermonthica* in northern Ghana with potential as biocontrol agents. *Biocontrol Sci Technol* **5**:157–161 (1995).

17 Sun Z, Matusova R and Bouwmeester H, Germination of *Striga* and chemical signalling involved: a target for control methods, in *Integrating New Technologies for Striga Control: Towards Ending the Witch-hunt*, ed. by Ejeta G and Gressel J. World Scientific, Singapore, pp. 47–60 (2007).

18 Hebbar KP, Lewis JA, Poch SM and Lumsden RD, Agricultural by-products as substrates for growth, conidiation and chlamydospore formation by a potential mycoherbicide, *Fusarium oxysporum* strain EN4. *Biocontrol Sci Technol* **6**:263–275 (1996).

19 Yonli D, Traoré H, Hess DE, Abbasher AA and Boussim IJ, Effect of growth medium and method of application of *Fusarium oxysporum* on infestation of sorghum by *Striga hermonthica* in Burkina Faso. *Biocontrol Sci Technol* **14**:417–421 (2004).

20 Berner DK, Windslow MD, Awad AE, Cardwell KF, Mohan Raj DR and Kim SK, *Striga Research Methods – a Manual*, 2nd edition, *Striga* Research Group. International Institute of Tropical Agriculture, Ibadan, Nigeria, 89 pp. (1997).

21 *SAS Software, Release 9.1.3.* SAS Institute, Cary, NC (2003).

22 Gomez AK and Gomez AA, *Statistical Procedures for Agricultural Research*, 2nd edition, Wiley-Interscience, New York, NY, 680 pp. (1984).

23 Gracia-Garza JA and Fravel DR, Effect of relative humidity on sporulation of *Fusarium oxysporum* in various formulations and effect of water on spore movement through soil. *Phytopathology* **88**:544–549 (1998).

24 Suzuki Y, Kono Y, Inoue T and Sakurai A, A potent fungal benzoquinone in etiolated sorghum seedlings and its metabolites. *Phytochemistry* **47**:997–1001 (1998).

25 Rich PJ and Ejeta G, Biology of host–parasite interactions in *Striga* species, in *Integrating New Technologies for Striga Control: Towards Ending the Witch-hunt*, ed. by Ejeta G and Gressel J. World Scientific, Singapore, pp. 19–32 (2007).

26 Ocamb CM and Kommendahl T, Rhizosphere competence of *Fusarium* species colonizing maize roots. *Phytopathology* **84**:166–172 (1994).

27 Ransom JK, Eplee RE, Langston MA and Norris RE, Methodology for establishing witchweed (*Striga asiatica*) in research plots. *Weed Technol* **4**:581–584 (1990).

28 Kanampiu F, Diallo A, Burnet M, Karaya H and Gressel J, Success with the low biotech of seed-coated imidazolinone-resistant maize, in *Integrating New Technologies for Striga Control: Towards Ending the Witch-hunt*, ed. by Ejeta G and Gressel J. World Scientific, Singapore, pp. 145–158 (2007).

29 Sands DC and Pilgeram AL, Methods for selecting hypervirulent biocontrol agents of weeds: why and how? *Pest Manag Sci* **this issue** (2009).

30 Kolombia G, Effect des acides amines (leucine, threonine et tyrosine) sur la germination de Benth. (Scrophulariacece) et leurs impacts sur lefficucite des agents delutte biologique au Benin, *MSc Thèse,* Université de Lome (2006).

31 Ejeta G, The *Striga* scourge in Africa: a growing pandemic, in *Integrating New Technologies for Striga Control: Towards Ending the Witch-hunt*, ed. by Ejeta G and Gressel J. World Scientific, Singapore, pp. 3–16 (2007).