Field evaluation of an integrated *Striga hermontica* management in Sub-Saharan Africa: Synergy between *Striga*-mycoherbicides (biocontrol) and sorghum and maize resistant varieties

B. Schaub\(^1\), P. Marley\(^2\), A. Elzein\(^1\)*, J. Kroschel\(^3\)

\(^1\) Institute for Plant production and Agroecology in the Tropics and Subtropics (380), University of Hohenheim, D-70593, Stuttgart, Germany, e-mail: gasim@uni-hohenheim.de

\(^2\) Institute for Agricultural Research (IAR), Ahmadu Bello University, P.M.B.1044, Samaru, Zaria, Nigeria, e-mail: psmarley@yahoo.co.uk

\(^3\) Integrated Crop Management Division, International Potato Center (CIP), Av. La Molina 1895, Apartado 1558, Lima 12, Peru, e-mail: j.kroschel@cgiar.org

*Corresponding author

Summary
The root parasite *Striga hermontica* (Del.) Benth. constitutes a major biotic constraint to staple food production in Africa. *Fusarium oxysporum* Schlecht. (Foxy 2 & PSM197) proved to be highly virulent against their target weed *S. hermontica*, host specific and they can be mass-produced. Thus, the antagonists offer a good prospect for *Striga* control in the future when incorporated into a long-term integrated *Striga* control program. This research focused on the development of an appropriate mycoherbicidal formulation, thereby reducing the amount of inoculum required for a practical field application.

“Pesta” granules of both isolates were made by encapsulating their chlamydospore-rich biomass in a matrix composed of durum wheat-flour, kaolin and sucrose. Their efficacy in combination with *Striga* resistant and susceptible sorghum (*Sorghum bicolor* (L.) Moench) and maize (*Zea mays* L.) cultivars was tested under field conditions at two locations (Samaru & Bagauda) in Nigeria. 2.0 g of “Pesta” granules of each isolate were applied per planting hole in which 5 g of *Striga* seed-sand (1:20 w/w) were inoculated. Both granular mycoherbicides (Foxy 2 & PSM197) were very effective with same potential, in controlling *Striga* on both susceptible and resistant maize and sorghum cultivars tested. Cumulatively (on average), they reduced the number of emerged *Striga* plants per plot by 75.3 %, *Striga* dry weight by 74.4 %, *Striga* flowers by 83.6 %, and crop plant infested by 64.8 % compared to the controls. The resistant maize and sorghum cultivars enhanced clearly both mycoherbicides efficacy. For maize, the reduction in all *Striga* parameters assessed compared to the controls was clear and in most cases significant for both isolates while for sorghum the differences were small. When the different treatment x cultivar combinations were compared, the combination Pesta granules x resistant cultivar had the strongest suppressive effect on *Striga*, especially for maize. The performance (growth, stalk dry weight & grain yield) of host plants sorghum and maize was slightly improved but not significantly compared to the controls. However, the significant reduction in *Striga* emergence and flowering as a result of combining mycoherbicides and host plant resistance is an important feature to prevent further *Striga* distribution and infestation. These findings are highly relevant to the realization of an integrated *Striga* control approach adoptable and applicable by subsistence farmers in Africa.

Keywords: Biocontrol, Mycoherbicide, Striga resistance, *Fusarium oxysporum*, Parasitic weed, *Striga hermontica*
Zusammenfassung
Ein integrierter Ansatz zur Bekämpfung von Striga hermontica in Afrika: Auswertung eines Mykoherbizids in Kombination mit resistenten Wirtspflanzen im Feld


Stichwörter: Biologische Bekämpfung, Mykoherbizid, Striga Resistenz, Fusarium oxysporum, Parasitische Unkraut, Striga hermontica

Introduction
Parasitic weeds of the genus Striga are considered to be the largest single biotic constraint to food production in Africa (Sauerborn 1991). They deprive water, nutrients and organic solutes from their host and further influence host physiology by causing depression of photosynthesis, as most obvious effect. Therefore, yield is reduced when crops are infested with Striga (Gurney et al. 2000). In West Africa, 48 % of the grain fields were found to be infested with Striga with an average yield loss of 24 % in six countries surveyed (Sauerborn 1991). Among Striga spp., S. hermontica was found to be the most widely distributed species in West Africa causing severe losses in maize, sorghum and millet (Pennisetum americanum L.) (Efron et al. 1989). Several methods have been developed for Striga control like crop rotation (Oswald and Ransom 2001), cultivation of Striga resistant varieties (Lagoke et al. 2000), deep planting and no tillage (Van Delft et al. 2000), herbicide seed coating in combination with herbicide resistant maize (Kanampu et al. 2003) or transplanting (Oswald et al. 2001). Various methods have been combined and tested as integrated Striga control approaches (Lagoke et al. 1994, Kuchinda et al. 2003, Schulz et al. 2003, Aliyu et al. 2004), all of them were successful to some extend but are not feasible for every farming system. However, on farm level, the management of Striga is still unsatisfactory and for many cases, a solution is still needed.

F. oxysporum showed potential to control S. hermontica effectively. Isolate Foxy 2, collected in northern Ghana by Abbaher et al. (1995), inhibited germination of Striga seeds in vitro completely, reduced Striga emergence by 98 % in pot trials and increased sorghum yield dramatically (Kroschel...
et al. 1996). It acts host specific (ELZEIN 2003) and does not produce phytotoxic compounds that present health risks (AMALFITANO et al. 2002). To reduce the amount of inoculum required a formulation named „Pesta“ was developed which reduced S. hermontica effectively in the glasshouse (ELZEIN 2003). Isolate PSM197, collected in the Northern Guinea Savannah of Nigeria by MARLEY et al. (1999), reduced S. hermontica in the field by 95 % when gritted sorghum grains were used as inoculum (MARLEY et al. 2004).

Control of Striga by soil application of a mycoherbicide and host plant resistance has several advantages. Striga resistant hosts are not immune but less infested by Striga compared to susceptible hosts. Among others a low germination stimulant production is often the most important resistance mechanisms. F. oxysporum attacks all Striga underground developmental stages including seeds (KROSCHEL et al. 1996). Therefore both reduce the Striga seed bank in the soil, prevent production of new seeds and increase the grain yield of the crop in the same cropping season. Additionally to their high potential in Striga control, they were assumed to be cost effective (ELZEIN 2003, GUPTA and LAGOKE 2000), require no changes in crop rotation and in case of the resistant host plants no additional labor. Therefore, they are assumed to fit into various farming systems.

However, high variation within and between populations of S. hermontica in different areas causes variability in resistance to Striga (KOYAMA 2000) and threatens durability of resistance. The approach including the mycoherbicide is supposed to make resistance more durable.

The objective of this study was to assess the efficacy of the granular formulation “Pesta”, containing F. oxysporum, under field conditions together with host plant resistance in an integrated approach. Two different isolates (Foxy 2 and PSM197) of F. oxysporum were used and their potential for control of S. hermontica was compared. The efficacy of this integrated Striga control approach was evaluated on maize and sorghum as host plants.

Materials and methods

Fungal isolates

Two isolates of F. oxysporum were used. Isolate Foxy 2 was collected by ABBASHER et al. (1995) in northern Ghana. Taxonomic identification was confirmed by the Federal Biological Research Center for Agriculture and Forestry, Berlin, Germany and the Commonwealth Mycological Institute, London, UK. Isolate PSM197 was collected by MARLEY et al. (1999) in Samaru, Nigeria. Taxonomic identification was confirmed by the International Mycological Institute (IMI), Egham, UK.

Inoculum mass production and formulation of mycoherbicides

Mass production was carried out using a certain type of submerged culture fermentation described by ELZEIN and KROSCHEL (2004). Thereby, F. oxysporum was grown in a suspension containing maize straw powder (< 0.5 mm) (0.5 % w/v) and wheat-based stillage (20 % v/v) to produce an inoculum consisting of a large amount of chlamydospores and microconidia and only few macroconidia. Concentration of chlamydospores, assessed using a hematocytometer, was always > 10⁷ spores per ml solution.

Solid “Pesta” granules were obtained using a process developed by CONNICK et al. (1991). For this study, 20 ml of liquid fungal inoculum of the isolates Foxy 2 or PSM197 were incorporated into a dough consisting of 32 g semolina (Rivella®, Italy), 6 g kaolin (Bolus, Roth®, Germany), 2 g sucrose (D(+) Saccharose, Roth®, Germany) and 3 ml of deionized water. To ensure equal distribution of the chlamydospores the dough was extruded and kneaded several times. Finally it was extruded using a manual pasta-maker to sheets that were 1-1.5 mm thick. These pasta-sheets were air-dried for three days at room temperature, ground using a Waring blender for ten seconds and sieved to obtain a particle size of 0.5-2 mm. The obtained Pesta granules were packed into plastic bags and stored in the fridge at 4 °C until use. Viability was checked immediately by dissolving 0.1 g of Pesta in 10 ml of water completely and plating 0.1 ml after serial dilution on PDA. Colony forming units (CFU/g) were counted after three days of incubation at 27 °C. The granules used in the experiment contained 1-4 x 10⁶ CFUs per g of Pesta.
Field evaluation of the efficacy of the combined mycoherbicide and \textit{Striga} resistant cultivars

Efficacy of PSM197 and Foxy 2 to control \textit{Striga} was evaluated in combination with \textit{Striga} resistant and susceptible cultivars of maize and sorghum. This results in 16 treatments, 2 isolates (PSM197 and Foxy 2) x 2 treatments (control and Pesta) x 2 crops (maize and sorghum) x 2 varieties (resistant and susceptible to \textit{Striga}). Resistant varieties used were Acr.97TZL Comp.1-W (maize) and ICSV111 (sorghum); susceptible ones were TZB-SR (maize) KSV4 (sorghum). \textit{Striga} seeds used for inoculation were collected in Samaru, Nigeria in the year 2003. The experiment was carried out during the rainy season from June until October in 2004. It was established as a randomized complete block design with three replications at two locations in Nigeria: Samaru (11°11’ N, 07°38’ E, altitude 686 m; Northern Guinea Savannah) and Bagauda (11°39’ N, 08°02’ E, altitude 500 m; Southern Sudan Savannah). Seeds of maize and sorghum were sown in ridges with a spacing of 0.75 m at a plot size of 15.75 m$^2$. Before sowing, each planting hole was inoculated with approximately 2 x $10^4$ \textit{S. hermontica} seeds and 2 g of Pesta, respectively. After establishment of the plants crops were thinned to two plants per stand. Weed control was carried out by hoe-weeding three weeks after sowing and by molding up the ridges (thereby weeds were covered with soil) six weeks after sowing. From now on, weeds were removed by hand-pulling only in order to avoid interactions with \textit{Striga} development. Fertilizer was applied at a rate of 120 kg N per hectare for maize and 64 kg N per hectare for sorghum. This amount was split into two equal parts of N. The first dose was applied in form of compound fertilizer N-P-K (20:10:10) three weeks after sowing; the second dose was applied in form of urea (46 % N) six weeks after sowing. For evaluation of \textit{Striga} control the number of \textit{S. hermontica} per plot, their flowers and their dry weight were assessed. Height, stalk and grain dry weight of the crop were taken at harvest and the \textit{Striga} damage on the crop was scored according to \textit{BERNER et al.} (1997).

All data were analyzed using SAS software, version eight (SAS Institute Inc., Cary, NC, USA). Data were subjected to the analysis of variance (ANOVA); normal distribution of the residues was checked using the Shapiro-Wilk Test. Data of all parameters was analyzed together for the two locations. Significant differences between the mean values were determined with Tukey’s HSD-Test at a level of $p \leq 0.05$. Results presented are the means of the original data of two experiments with the standard error of the means.

**Results**

Both granular mycoherbicides (Foxy 2 & PSM197) were very effective with same potential, in controlling \textit{Striga} on both susceptible and resistant maize and sorghum cultivars tested. No difference was observed between the impact of the two isolates of \textit{F. oxysporum} on \textit{S. hermontica}. Cumulatively (i.e. the average effect of both isolates in combination with resistant and susceptible cultivars), they reduced the number of emerged \textit{Striga} plants per plot by 75.3 %, \textit{Striga} dry weight by 74.4 %, \textit{Striga} flowers by 83.6 %, and crop plant infested by 64.8 % compared to the controls (Fig. 1, Tab. 1). The combination Pesta granules x resistant maize and sorghum cultivars enhanced clearly both mycoherbicides efficacy, and showed the strongest suppressive effect on \textit{Striga} compared to the susceptible cultivars. The difference between the resistant and the susceptible cultivars was stronger for maize than for sorghum. For maize, the reduction in all \textit{Striga} parameters assessed compared to the controls was clear and in most cases significant for both isolates while for sorghum the differences were small (Fig. 1, Tab. 1). On average, the reductions per plot in the number of emerged \textit{Striga} plants, \textit{Striga} dry weight, the number of infested maize plants, and the number of \textit{Striga} flowers, respectively, were 87 %, 81.4 %, 78.4 % and 83.5 %, when the two isolates integrated with the resistant maize cultivars (Fig. 1, Tab. 1). On the other hand, the respective reductions when the two isolates integrated with the susceptible maize cultivars were 87.7 %, 56.2 %, 58.1 % and 55 %.

Crop performance (height, grain yield, stalk dry weight and \textit{Striga} damage score assessed according to \textit{BERNER et al.} 1997) was slightly improved but not significantly in the treatments where Pesta was applied compared to the controls; resistant cultivars did not show higher performance than susceptible ones also (data not shown).
Fig. 1: Number of *Striga hermontica* plants per plot on maize (A) and sorghum (B) (*Striga* susceptible (S) and resistant (R) cultivars) after inoculation with Pesta formulations containing *Fusarium oxysporum* isolates (PSM197 or Foxy 2). Controls: (S) susceptible, (R) resistant cultivars without Pesta formulations. Values are the means of two experiments with three replications each. Vertical bars indicate the standard error of the means. Means with the same letter are not significantly different at $p \leq 0.05$ after Tukey’s HSD-Test.

*Abb. 1:* Anzahl von *Striga hermontica* in Mais (A) und Sorghum (B) (*Striga*-anfällige (S) und resistente (R) Sorten) pro Parzelle mit und ohne Anwendung von Pesta (enthält *Fusarium oxysporum*, Isolat PSM197 oder Foxy 2).
Tab. 1: Combined effect of Pesta mycoherbicides \(^\text{a), b)}\) and maize and sorghum susceptible (S) and resistant (R) cultivars on Striga hermontica infestation.

\[\text{Tab. 1: Wirkung von Pesta Mykoberbizids} \(^\text{ab})\) \text{in Kombination mit Striga-anfälligen (S) und resistenten (R) Wirtspflanzen auf Striga hermontica-Befall.}\]

\(\text{a)}\) Pesta produced with \textit{Fusarium oxysporum}, isolate Foxy 2

<table>
<thead>
<tr>
<th>Crop</th>
<th>Treatment</th>
<th>\textit{Striga dry weight, g}</th>
<th>\textit{Striga flowers, No.}</th>
<th>Crop plants infested, No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>Control (S)</td>
<td>35.2 (7.0) (\text{a)})</td>
<td>213.6 (90.5) (\text{a)})</td>
<td>10.1 (1.7) (\text{a)})</td>
</tr>
<tr>
<td></td>
<td>Control (R)</td>
<td>6.4 (2.4) (\text{ab})</td>
<td>7.8 (1.1) (\text{ab})</td>
<td>4.6 (0.1) (\text{ab})</td>
</tr>
<tr>
<td></td>
<td>Pesta (S)</td>
<td>16.3 (10.9) (\text{b})</td>
<td>96.6 (68.3) (\text{ab})</td>
<td>3.3 (0.9) (\text{ab})</td>
</tr>
<tr>
<td></td>
<td>Pesta (R)</td>
<td>1.1 (0.4) (\text{b})</td>
<td>2.6 (0.9) (\text{b})</td>
<td>1.1 (0.3) (\text{b})</td>
</tr>
<tr>
<td>Sorghum</td>
<td>Control (S)</td>
<td>24.5 (10.4) (\text{a)})</td>
<td>59.5 (35.0) (\text{a)})</td>
<td>4.0 (0.5) (\text{a)})</td>
</tr>
<tr>
<td></td>
<td>Control (R)</td>
<td>10.3 (2.7) (\text{ab})</td>
<td>27.4 (16.0) (\text{a})</td>
<td>3.3 (0.5) (\text{a})</td>
</tr>
<tr>
<td></td>
<td>Pesta (S)</td>
<td>3.8 (0.9) (\text{ab})</td>
<td>2.9 (2.0) (\text{a})</td>
<td>2.4 (0.3) (\text{a})</td>
</tr>
<tr>
<td></td>
<td>Pesta (R)</td>
<td>1.2 (0.2) (\text{b})</td>
<td>1.7 (1.2) (\text{a})</td>
<td>1.3 (0.4) (\text{a})</td>
</tr>
</tbody>
</table>

\(\text{b)}\) Pesta produced with \textit{Fusarium oxysporum}, isolate PSM197

<table>
<thead>
<tr>
<th>Crop</th>
<th>Treatment</th>
<th>\textit{Striga dry weight, g}</th>
<th>\textit{Striga flowers, No.}</th>
<th>Crop plants infested, No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>Control (S)</td>
<td>12.5 (3.0) (\text{a)})</td>
<td>52.9 (15.2) (\text{a)})</td>
<td>7.7 (1.1) (\text{a)})</td>
</tr>
<tr>
<td></td>
<td>Control (R)</td>
<td>1.5 (1.1) (\text{ab})</td>
<td>0.9 (0.7) (\text{b})</td>
<td>2.6 (1.6) (\text{ab})</td>
</tr>
<tr>
<td></td>
<td>Pesta (S)</td>
<td>5.2 (2.0) (\text{ab})</td>
<td>23.6 (9.0) (\text{ab})</td>
<td>3.9 (1.2) (\text{ab})</td>
</tr>
<tr>
<td></td>
<td>Pesta (R)</td>
<td>0.3 (0.3) (\text{b})</td>
<td>0.0 (0.0) (\text{b})</td>
<td>0.5 (0.3) (\text{b})</td>
</tr>
<tr>
<td>Sorghum</td>
<td>Control (S)</td>
<td>7.4 (2.9) (\text{a)})</td>
<td>21.1 (12.2) (\text{a)})</td>
<td>2.7 (0.4) (\text{a)})</td>
</tr>
<tr>
<td></td>
<td>Control (R)</td>
<td>4.0 (2.2) (\text{a)})</td>
<td>11.9 (8.4) (\text{a})</td>
<td>1.9 (0.5) (\text{ab})</td>
</tr>
<tr>
<td></td>
<td>Pesta (S)</td>
<td>0.6 (0.3) (\text{a})</td>
<td>0.0 (0.0) (\text{a})</td>
<td>0.2 (0.1) (\text{b})</td>
</tr>
<tr>
<td></td>
<td>Pesta (R)</td>
<td>1.7 (0.7) (\text{a})</td>
<td>0.0 (0.0) (\text{a})</td>
<td>0.9 (0.4) (\text{ab})</td>
</tr>
</tbody>
</table>

Controls: (S) susceptible, (R) resistant cultivars without Pesta formulations. Values are the means of two experiments each with three replications. Values in brackets indicate the standard error of the means. Means with the same letter are not significantly different for each parameter at \(p \leq 0.05\) after Tukey’s HSD-Test.

**Discussion**

Considering the constraints to successfully controlling parasitic weed \textit{Striga} so far, it is well recognized that no single method of control can provide effective and economically acceptable solution. Therefore, an integrated control approach is essential, ideal and useful to small-scale farmers, in order to achieve sustainable crop production. No standard integrated control package for \textit{Striga} can be suggested; therefore it needs to be adjusted to individual cropping systems, local needs and preferences. Host plant resistance would probably be the most feasible and potential method for \textit{Striga} control. Using biotechnological approaches (including biochemistry, tissue culture, plant genetics and breeding, and molecular biology), significant progress has been made in developing screening methodologies and new laboratory assays, leading to the identification of better sources of parasitic weeds hosts resistance (HAUSSMANN \textit{et al.} 2000; OMANYA 2001; EJETA 2002). Considerable progress has been achieved by INTSORMIL, IITA, and by CIMMYT in developing (identifying) several \textit{Striga} resistant sorghum and maize varieties that are used nowadays in various parts of Africa (Kim 1994; LANE \textit{et al.} 1997; EJETA 2002). Biological control, especially using fungal antagonists against \textit{Striga}, has gained considerable
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attention in recent years and appears to be promising as a viable supplement to other control methods. Many mycoherbicide candidates against Striga are still in the developmental stage, including evaluation of formulations and delivery. Therefore, the next step would have to consider how to integrate these biocontrol agents in different crop management systems.

One important feature of a successful mycoherbicide is the availability of a suitable inoculum for application in the field. The formulation tested (Pesta) showed a high level of Striga control in the field at a rate of 40 kg per ha when applied directly into the planting hole. This way of application is supposed to be feasible as local farmers do sowing manually and the amount of inoculum is not too high. The development of biocontrol products that are easily delivered such as Pesta formulation could accelerate the acceptance of these products. Additional advantages of Pesta formulations are: can be produced on a large scale, convenient to store (Elzein et al. 2004), simple to use, compatible with existing agricultural machinery, and can be easily integrated with existing Striga control methods, e.g. use of resistant varieties.

Integrated Striga control approaches are most attractive when they reduce Striga infestation on long term and increase crop yield already in the season of application. In this study, both isolates (PSM197 and Foxy 2) of F. oxysporum mycoherbicide as well as host plant resistance inhibited emergence and flowering of S. hermontica in the field on maize and sorghum effectively. However, crop yield was not improved significantly. The reason might be that Striga infestation in the experiment was very low in general, only 16% of the crop plants were infested in the control and yield was therefore hardly reduced. At a higher level of Striga infestation effects on crop performance might occur. Nevertheless, the significant reduction in Striga emergence and flowering as a result of combining mycoherbicides and host plant resistance is an important feature to prevent further Striga distribution and infestation in the fields as seeds remain viable in the soil for at least up to 14 years (Parker and Riches 1993).

Regarding this, the approach of combining host plant resistance and the mycoherbicide might be a good option for farmers and should be tested on farmers’ fields. Indeed, it is important to choose the best adapted resistant cultivar for every location as resistance is often regional and also performance depends on local agro ecological conditions. Besides this, further work will be carried out to develop a seed treatment with the mycoherbicide in order to further reduce the amount of the inoculum required as well as labor during application.

In conclusion, the efficacy of Fusarium oxysporum, formulated as Pesta granules for control of Striga hermontica has been proved under field conditions. The integrated approach combining the mycoherbicide with host plant resistance was successful as the two control methods had an additive effect. These findings are highly relevant to the realization of an integrated Striga control approach adoptable and applicable by subsistence farmers in Africa.

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References


