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Use of *Fusarium oxysporum* for the control of *Striga hermonthica* in maize (*Zea mays* L.)

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ABSTRACT
Objective: To evaluate the efficacy of a granular mycoherbicide formulation of *Fusarium* sp. for the control of *Striga hermonthica* in maize under a field condition.
Methodology and results: Two maize varieties (Across 97 TZL and a farmer's local) and four treatments (*Fusarium oxysporum* followed by 2,4 – D, *F. oxysporum* fb supplementary hoe weeding (SHW), *F. oxysporum* fb Triclopyr and a control (hoe weeded at 3 and 6 weeks after sowing) were laid out in a split – plot design with three (3) replications; assigning varieties to the main – plots and weed control treatments to the sub – plots. This trial was carried out in two locations (Makurdi and Lafia). Generally, number of plants infected with *Striga* and shoot count was highest in Makurdi than Lafia. Variety Across 97 TZL had significantly lower/fewer number of plants infected at 9 and 12 Weeks after sowing (WAS) and also delayed the emergence of *Striga* to 49 days after sowing (DAS), while the farmers' local initiated early emergence at 36 DAS. Among the *Striga* control treatments, *F. oxysporum* either fb post emergence (POE) 2, 4 – D or Triclopyr at the rate of 0.36kg a.i/ha each resulted in significantly fewer plants infested by the parasitic plant and consequently higher grain yields than those fb by SHW or hoe weeded check.
Conclusions and application of findings: The study results demonstrate the high potentiality of using *F. oxysporum* (mycoherbicide) for the control of *S. hermonthica* by spot application at sowing and thereafter followed by either 2, 4 –D or Triclopyr POE at 6 WAS. The use of maize grits which is readily available to propagate *F. oxysporum* makes it quite cheap for local farmers instead of the use of potato dextrose agar.

Key words: biocontrol, *Striga hermonthica*, *Fusarium oxysporum*, mycoherbicide, maize.

INTRODUCTION

Parasitic weeds of the genus *Striga* (Scropulariaceac) strongly affect host crops such as maize (*zea mays* L.), sorghum (*Sorghum biocolar* (L.) Moench), pearl millet *pennisetum americanum* (L.) Beeke), rice (*Oryra sativa* L.) and cowpea (*Vigna unguiculata* (L.) Walpers). as a consequence, these weeds are important growth – reducing factors in crops in vast areas of the savannah zone in Africa (Parker & Riches, 1993). Of all *Striga* species, *Striga hermonthica* (Del.) Benth is the most economically important parasitic weed in the Nigerian Savanna (Emechebe *et al*., 1991; Lagoke *et al*., 2000; Kuchinda *et al*., 2003; Marley *et al*., 2004). Yield reduction caused by *S. hermonthica* can be up to 79% even under good management (Lagoke *et al*., 1997). One of the reasons why Striga has such devastating impacts on the growth and yield of cereals relates to its dual mode of action. First, Striga plants compete effectively with the host for carbon, nitrogen and inorganic solutes (Frost *et al*., 1997; Gurney *et al*., 1995). Secondly, the parasite has a so – called 'phytotoxic' effect on the host plant within days of attachment (Berner *et al*., 1995; Frost *et al*., 1995; Gurney *et al*., 1995). A very small parasite biomass, with attachments of less than 4mm in size, results in a large reduction in host height, biomass and eventual grain yield (Gurney *et al*., 1995). The mechanism underlying the 'phytotoxic' effect has not yet been elucidated, but may involve the production of a 'toxin' (Musselman & Press, 1995).

Several approaches for controlling *S. hermonthica* in cereals have been investigated including host plant resistance (Ramaiah, 1991; Lagoke, *et al*., 2000; Adeosun *et al*., 2001; Gwary *et al*., 2001); cultural and mechanical practices such as hand – pulling, crop rotation, trap – cropping, mixed cropping, nitrogen fertilization, tillage and planting methods (Lagoke *et al*., 1991; Kureh *et al*., 1999; Kuchinda *et al*., 2003); chemical herbicides (Ariga & Berner 1993; Bagonneaud – Berthome *et al*., 1995) and biological control (Onu *et al*., 1996; Marley *et al*., 1999; Nekoum &Marley 2002). The potential of using fungi for biological control of *Striga* was proposed by Musselman (1983) and has since generated research interest. Adeoti (1993) and Weber *et al*. (1995) have shown than *Fusarium equiseti*, Cercospora, Fusarium, Phoma, Alternaria and *Macrophomina* spp. are associated with *Striga*. The potential of *Fusarium oxysporum* grown in grain sorghum as a biocontrol agent for the control of *S. hermonthica* has been reported (Krocshel *et al*., 1996; Abbasher *et al*., 1998; Marley *et al*., 1999; Marley & Shebayan, 2005). Isolates of *Fusarium oxysporum* recovered from *S. hermonthica* have been shown to infect *Striga* spp and no other crops or vegetables (Abbasher *et al*., 1998; Elzein, 2003; Marley & Shebayan, 2005).

The development of *Fusarium oxysporum* into a mycoherbicide in liquid and/or granular formulation and their evaluation for efficacy under screen house and field conditions have been reported (Hess *et al*., 2002). The purpose of this study was therefore to evaluate the efficacy of the mycoherbicide formulation under field conditions.

MATERIALS AND METHODS

Preparation of pathogenic fungi (mycoherbicide): Biological control using *F. oxysporum* (isolate PSM 197) as mycoherbicide was reported (Marley *et al*., 1999, 2000; Marley & Shebayan, 2005). *Fusarium oxysporum* (isolate PSM 197) was isolated from *S. hermonthica* stems and single spore isolates made into stock cultures (Marley and Shebayan, 2005). The isolates are maintained on potato dextrose agar (PDA) amended with streptomycin (Difco) and stored in the refrigerator at 4^0C Fresh starter cultures are made when required. The biocontrol agent was produced on gritted maize grains (whole grain broken into smaller pieces) in the laboratory as described by Marley *et al*. (1999). Gritted grain (500g) was placed in 1L flat – bottomed flasks each containing 250ml of sterile distilled water. Flasks were shaken to ensure that the substrate was properly moistened and excess water was poured off prior to autoclaving for 1h at 121^0C (103.5kPa). After cooling, each flask was inoculated with three agar plugs (5mm diameter) of the isolate and then incubated at 28^0C for 7 days. During the incubation period, each flask was shaken daily (10 hrs) to allow for full colonization of the grains by the pathogen. Colonized grains were harvested 14 days after inoculation and stored in refrigerator at 4^0C for use when required as the mycoherbicide.

Field evaluation: The field experiments were conducted in 2007/2008 wet seasons at the teaching and research farm of the University of Agriculture, Makurdi (07^0 14'N, 08^0 37'E) and the Model Extension Village, Danka – Sarki, Lafia (08^0 3'N and 07^0 31'E) in the Southern Guinea Savanna of Nigeria. The two sites were naturally and heavily infested with *Striga hermonthica*.

The trials were established at Makurdi and Lafia on 28^{th} May and 16^{th} June, 2008, respectively. The two sites of the trials were ploughed, harrowed and ridged at 0.75m apart. Two maize varieties (Across 97 TZL and a local) were planted 50cm apart for each cultivar. In each planting hole, 2g (53.33 kg/ha) of mycoherbicide in each treatment was applied pre – sowing. Four treatments were used as follows: (1) *F.*

oxysporum followed by (fb) 2, 4 – D; (2) *F. oxysporum* fb supplementary hoe weeding (SHW); (3) *F. oxysporum* fb Triclopyrand (4) a control (No *F oxysporum*, but hoe weeded). The trials were planted in a split – plot design with three replications. The two maize varieties formed the main plot treatments while the *Striga* control methods were the sub – plot treatments. The gross and net plot sizes were $9m^2$ and $4.5m^2$ (4 ridges and 2 ridges of 3m length each), respectively. Spot application of fertilizer was carried out at 120kg N/ha, 60kg P_2O_5/ha and 60kg K_2O/ha to maize using 15 – 15 – 15 N-P-K compound fertilizer at 3 WAS and later top-dressed with Urea at 6 WAS. The post emergence herbicides (2, 4 – D and Triclopyr) were applied at 6 WAS (at about 20% *Striga* infestation) sing a knapsack sprayer (CP_3) with spray volume of 250L/ha.

Data recording and analysis: Observations made included number of maize plants infected by *Striga*, *Striga* shoot count per unit area at (9WAS, 12WAS and 20WAS), days to first *Striga* emergence, weight of 1000 grains and grain yield. Data were subjected to analysis of variance and means compared using Least Significant Difference (LSD) at 5% level of probability.

RESULTS AND DISCUSSION

In the trials, locations, varieties and *Striga* control methods differed significantly with respect to the number of maize plants infected at 9, 12 WAS and 20WAS (Table 1). Generally, the number of maize plants infected with *Striga* was highest throughout the period of observation in Makurdi than at Lafia. Variety Across 97 TZL had significantly less plants infected at 9 and 12 WAS than the local variety, but at 20 WAS Across 97 TZL recorded higher plants infected by the parasitic plant than the local variety. Throughout the period of observation, the hoe weeded check, had significantly higher number of plants infected when compared to other *Striga* control methods. At 9 WAS, the minimum number of maize plants infected was obtained with treatments that received *F. oxysporum* followed by POE Triclopyr at 0.36kg a.i/ha. . However, at 12 WAS and at 20WAS, number of maize plants infected was not significantly different from treatments that received *F. oxysporum* followed by POE 2, 4 – D at the rate of 0.36kg a.i/ha. This observation is similar to previous reports on use of a *Fusarium* – based mycoherbicide for control of *Striga* under field conditions. They obtained a complete inhibition of *S. hermonthica* emergence when a *chlamydospores* powder was added to the soil at sowing or when sorghum seeds coated with *chlamydospores* were sown. An inoculum production strategy based on a cottage industry model that utilizes a liquid fermentation method with sorghum straw as the substrate has been proposed. Arabic gum, another in – expensive, locally available material was used to stick the *Fusarium* spores on to sorghum seeds prior to planting (Marley *et al*., 2004). Marley *et al.* (2004) revealed that treatment of plots with mycoherbicides at sowing by spot application of 5g of *Fusarium* – colonized grains in each planting hole, equivalent to 165kg/ha was very effective *S. hermonthica*.

Locations and varieties differed significantly in respect to the number of days to *Striga* emergence (Table 1). Makurdi initiated early *Striga* emergence at 30 days after sowing (DAS) as compared to Lafia at 46 DAS. The obsereved difference may be due to environmental effects, e.g. temperature, water or presence of other stimulant in the soil that was more favourable for the germination of Striga seeds for Makurdi than in Lafia. Maize variety Across 97 TZL delayed the emergence of *Striga* (49 DAS), while the local variety initiated early emergence of *Striga* (36 DAS). However, weed control methods did not have significant effect on days to *Striga* emergence, but the trend indicated early emergence in hoe weeded check plots (38 DAS). It has been reported that pre – planting inoculation of the *Fusarium* pathogen inoculum caused between 7 and 14 days delay in *Striga* emergence and consequently a significant reduction in *S. hermonthica* population (Marley *et al*., 2005).

Table 1: Effect of F. oxysporum and post – emergence herbicide on number of plants infected, the time of emergence of Striga at Makurdi and Lafia, 2008 wet season.

Treatments	Number of days to first Striga emergence	Number of plants infected/4.5m²		
Location		**9WAS**	**12WAS**	**20WAS**
Makurdi	39[b]	8.50[a]	8.67[a]	8.83[a]
Lafia	46[a]	2.62[b]	3.00[b]	3.17[b]
LSD	4.95	1.24	1.56	1.09
Variety				
Across 97 TZL Comp 1 – W	49[a]	4.62[b]	4.92[b]	6.92[a]
Local	36[b]	6.50[a]	6.75[a]	5.58[b]
LSD	6.66			
Striga control methods				
F oxysporum fb[1]	46	4.50[c]	4.92[bc]	5.00[c]
F oxysporum fb[2]	43	5.33[b]	5.67[b]	6.00[b]
F oxysporum fb[3]	41	3.92[d]	4.17[c]	4.25[c]
Hoe weeded (check)[4]	38	8.50[a]	8.58[a]	8.75[a]
LSD	10.82	0.96	1.06	0.96
Interactions				
Loc. X Var.	NS	NS	NS	NS
Loc. X Wcm.	NS	NS	NS	NS
Var X Wcm	NS	NS	NS	NS
Loc. X Var. X Wcm	NS	NS	NS	NS

Means in a column of any set of treatments followed by different letters are not significantly different at 5% level using LSD. 1 = POE 2, 4 – D (0.36kga.i/ha) at 6 WAS; 2 = POE SHW at 6 WAS; 3 = POE Triclopyr (0.36kga.i/ha) at 6 WAS; 4 = At 3 and 6 WAS.

There was a significant difference in *Striga* shoot count at 9, 12 WAS and at 20 WAS in respect of locations, varieties and weed control methods (table 2). Lafia had significantly lower *Striga* shoot count than Makurdi throughout the period of observation in this trial. The local maize variety had significantly higher *Striga* shoot count than cv. Across 97 TZL throughout the period of observation. In the context of this paper "resistant" refers to host cultivars that are less attacked and thus have less damage and number of emerged *Striga* plants (Parker & Riches, 1993) Plants of cultivar Across 97 TZL, being a resistant variety, have been reported to produce lower amounts of germination stimulants in their root exudates, leading to smaller numbers of attached parasites and/or to later attachment of the parasites to the host (Gurney *et al*., 2002).

Parker and Riches (1993) suggested three ways by which crop cultivars resist *Striga* attack. These include mechanical barrier whereby the host root cells prevent the Striga haustoria from attaching, anti – haustoria initiation factors, and low stimulant production. The mechanism of resistance of cv. Across 97 TZL is not documented but may be through reduced production of germination stimulant since fewer *Striga* plants were recorded. I therefore recommend further investigation into the mechanism of resistance in this cultivar. In *Striga* control treatments, hoe weeded check at 9, 12 WAS and 20WAS had significantly higher *Striga* shoot count, followed by those treated with *F. oxysporum* followed by SHW. The least *Striga* shoot count was obtained with treatments of *F. oxysporum* followed by POE 2, 4 – D at the rate of 0.36kg a.i/ha. This result is in accordance with previous findings in which the isolate *Fusarium oxysporum* (Foxy 2) was able to reduce the germination of *S. hermonthica* seeds by more than 90% when the fungus was applied during the seed–conditioning phase, and it prevented the emergence by 98% when it was used as soil inoculum (Kroschel *et al*., 1996). This was also the case for *F. nygamia* attacking *S. hermonthica* on sorghum and pearl millet in West Africa (Abbasher, 1994). It is assumed that the reduction in seed germination and death of the germinated seedsbefore they attach, due to foxy 2, led to the reduced number of emerged *S. hermonthica* as well. Thus foxy 2 exerts its effect by destruction of the seeds and prevention of emergence and subsequent reproduction.

Table 2: Effect of F. oxysporum and post – emergence herbicides on Striga shoot count, weight of 1000 grains and grain yield at Makurdi and Lafia, 2008 wet – season.

Treatments	Striga shoot count/4.5m^2			Weight of 1000 grains (g)	Grain yield (kg/ha)
Location	**9WAS**	**12WAS**	**20WAS**		
Makurdi	13.58[a]	18.42[a]	20.96[a]	265	1415[b]
Lafia	3.71[b]	5.96[b]	8.80[b]	263	2721[a]
LSD	4.69	2.18	0.17	22.46	45.70
Variety					
Across 97 TZL Comp 1 – W	7.17[b]	9.12[b]	10.08[b]	255[b]	2474[a]
Local	10.12[a]	15.17[a]	1975[a]	271[a]	1662[b]
LSD	1.62	4.10	0.60	7.93	477.4
Striga Control Methods					
F. oxysporum fb[1]	5.92[c]	10.42[bc]	11.42[c]	275[a]	2387[a]
F. oxysporum fb[2]	8.83[b]	10.75[b]	16.00[b]	256[b]	1991[c]
F. oxysporum fb[3]	4.75[c]	8.50[c]	9.75[d]	269[a]	2773[b]
Hoe weeded (check)[4]	15.08[a]	19.08[a]	22.50[a]	253[b]	1121[d]
LSD	1.46	2.16	1.02	11.52	250.80
Interactions					
Loc X Var	NS	NS	*	NS	NS
Loc X Wcm	*	*	NS	NS	NS
Var X Wcm	NS	NS	*	NS	NS
Loc X Var X Wcm	NS	NS	N	NS	NS

Means in a column of any set of treatments followed by different letters are not significantly different at 5% level using LSD. 1 = POE 2, 4 – D (0.36kga.i/ha) at 6 WAS; 2 = POE SHW at 6 WAS; 3 = POE Triclopyr (0.36kga.i/ha) at 6 WAS; 4 = At 3 and 6 WAS.

There were significant interactions between location x weed control methods at 9 and 12 WAS; location x variety and variety x weed control methods at harvest and number of *Striga* shoot count (Table 3, 4 and 5). At 9 WAS and 12 WAS, the use of hoe weeded check at Makurdi gave the highest *Striga* shoot count, while the lowest were obtained in Lafia that received *F. oxysporum* followed by either 2, 4 – D or Triclopyr each at 0.36kg a.i/ha which was not equally statically different from those *F. oxysporum* followed by SHW (Table 3) at harvest, of the two location, variety local at Makurdi increased *Striga* shoot count while the least was recorded in Lafia with Acorss 97 TZL (Table 4). In the variety x weed control method interaction, Across 97 TZL and *F. oxysporum* fb POE Triclopyr at the rate of 0.36kg a.i/ha depressed *Striga* shoot count the most, while the local variety that received hoe weeded check gave the highest *Striga* count (Table 5). This work agrees with earlier work by Kureh *et al.* (2003) who reported that *Striga* had a detrimental effect on the development and productivity of local susceptible variety, but infestation did not lower the productivity of Across 97 TZL as it supported fewer *Striga* shoots and consequently suffered less damage than most commercial and farmers' cultivars.

Table 3: Interaction between location and *Striga* control methods on *Striga* shoot at 9 and 12 WAS, 2008 wet season.

Striga Control Methods	Location (9WAS)		Location (12WAS)	
	Makurdi	**Lafia**	**Makurdi**	**Lafia**
F. oxysporum fb[1]	9.17[c]	2.67[de]	16.50[b]	4.33[ef]
F. oxysporum fb[2]	14.83[b]	2.83[d]	16.00[bc]	5.50[e]
F. oxysporum fb[3]	8.00[c]	1.50[de]	14.00[bc]	3.00[ef]
Hoe weeded (check)[4]	22.33[a]	7.83[c]	27.17[a]	11.00[d]
LSD (P<0.05)	3.55		2.84	

Means in a column of any set of treatments followed by different letters are not significantly different at 5% level using LSD. 1 = POE 2, 4 – D (0.36kga.i/ha) at 6 WAS; 2 = POE SHW at 6 WAS; 3 = POE Triclopyr (0.36kga.i/ha) at 6 WAS; 4 = At 3 and 6 WAS.

Locations did not differ significantly with respect to weight of 1000 grains. However, varieties and *Striga* control methods had significant effect (Table 2). The local variety resulted in significantly heavier grains than Across 97 TZL. This can be said to be attributable to varietal difference. Among the weed control treatments, *F. oxysporum* either fb FOE 2, 4 – D compared to *F. oxysporum* fb SHW and hoe weeded check.

Table 4: Interaction of location and variety on Striga shoot count at harvest, 2008 wet season

Variety	Location	
	Makurdi	**Lafia**
Across 97	14.58^{b}	5.58^{d}
Local	27.33^{a}	12.17^{c}
LSD (P<0.05)	1.65	

Means in a column of any set of treatments followed by different letters are not significantly different at 5% level using LSD.

Table 5: Interaction of variety and weed control methods on Striga shoot count at harvest at Makurdi and Lafia, 2008 wet season.

Weed Control Methods	Variety	
	Across 97	**Local**
F. oxysporum fb1	8.17^{ef}	14.67^{cd}
F. oxysporum fb2	9.83^{e}	22.17^{b}
F. oxysporum fb3	6.00^{f}	13.50^{d}
Hoe weeded (check)4	16.33^{c}	28.67^{a}
LSD (P<0.05)	2.83	

Means in a column of any set of treatments followed by different letters are not significantly different at 5% level using LSD. 1 = POE 2, 4 – D (0.36kga.i/ha) at 6 WAS; 2 = POE SHW at 6 WAS; 3 = POE Triclopyr (0.36kga.i/ha) at 6 WAS; 4 = At 3 and 6 WAS.

This could be attributed to the fact less number of *Striga* infestation and shoot count was observed/recorded by those treatments that gave heavier grains.

In the trials, location, variety and *Striga* control methods differed significantly with respect to grain yield (Table 2). Lafia produced the highest grain yield (2721kg/ha) when compared to that of Makurdi (1415kg/ha). The low yield at Makurdi may be due to more seeds of the parasitic plant in the soil to contend with than that of Lafia. The variety Across 97 TZL produced higher grain (2474kg/ha) than that of the local (1662kg/ha). This result confirmed earlier reports that improved open – pollinated (op) maize varieties and hybrids are less damaged owing tolerance to *Striga*. They have been reported to produce higher grain yields than the susceptible cultivars and/or farmers' local maize varieties (Kim 1994; Berner *et al*., 1999). The use of *F. oxysporum* followed by either POE 2, 4 – D or Triclopyr each at 0.36kg a.i/ha resulted in significantly higher maize grain yield than those followed by SHW or hoe weeded check. The low yield in the hoe weeded check can be attributed to higher level of *Striga* infestation and shoot count in those plots. The higher grain yields obtained with treatments that received post emergence (POE) application of either 2, 4 – D or Triclopyr confirmed earlier work when 2, 4 – D alone, mixture with diflufenican, Triclopyr resulted in higher grain yield than the control (Lagoke *et al*., 1993).

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