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Striga hermonthica reduction using *Fusarium oxysporum* in Kenya

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The biological control agent *Fusarium oxysporum* f.sp. *strigae* isolate Foxy 2 had shown potential as a biological control in West Africa, however it failed to achieve the same results when used on Kenyan *Striga hermonthica*. A *F. oxysporum* isolate FK3 was obtained from infected *S. hermonthica* in a maize crop in Kenya and tested as a potential control agent of *S. hermonthica* in Kenya. Two pot trials were conducted where the *S. hermonthica* seed and varying rates of isolate FK3 (7.5 to 60 × 10^7 CFUs per pot) were added prior to planting of maize. Numbers of *S. hermonthica* plants were reduced significantly at the lowest rate of FK3 application when compared to the control from 10.7 to 5.5 per pot in the first trial and 21.3 to 7.9 plants per pot in the second trial. Where a more susceptible variety of maize was used in the second trial evidence of improved maize growth (fresh root mass and stover weight) and yield (weight of grains and whole cob weight) was associated with the reduction in *S. hemonthica* numbers. More extensive field trials are recommended to fully assess the impact of FK3 on maize yield. The findings support the idea that regional genetic variation in both *S. hermonthica* and the pathogen *F. oxysporum* may explain the regional specificity of *F. oxysporum* isolates as a potential biological control agents.

Key words: Biological control, maize, mycoherbicide, parasitic plants, striga, *Fusarium*.

INTRODUCTION

Maize is grown throughout the world but there are large differences in yield and consumption. African countries are relatively small producers in total tonnage, for instance the USA and China are the largest producers with 274 and 208 million MT/year while South Africa, with 12 million MT/year is the largest African producer. However consumption as measured in g/person/day is highest in Africa with six out of the top ten consuming countries being in Africa (Ranum et al, 2014). Maize is by far the most important food crop in Kenya, playing an integral role in national food security. Maize is the primary staple food for most Kenyans, accounting for 36% of all calories consumed and 65% of staple food calories consumed. Kenya produces around 3 million tonnes of maize per year on about 2 million ha of land annually (Short et al., 2012). *Striga hermonthica* (Del) Benth (Witch weed) is a wide spread and endemic parasitic weed of a wide range of gramineous species, including

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maize, sorghum, millet and rice. The weed is found across sub-Saharian Africa (Beed et al., 2007). In East Africa, *S. hermonthica* is found around the Lake Victoria basin in Kenya, Tanzania and Uganda. In Kenya, *Striga* infestation is most severe in Nyanza and Western provinces. The parasitic weed is found in about 75,000 hectares of farmland and results in crop losses estimated at about US$ 10 to 38 million per annum (Manyong et al., 2008). *S. hermonthica* is a major cause of yield loss in Western Kenya in maize where up to 70% losses have been reported (Khan et al., 2008). Despite extensive research and extension efforts the problem of this parasitic weed has not receded and with poor farming practices and potential climatic change the problem may further increase (Jamil et al., 2012).

There are numerous control strategies that have been developed for the management of *S. hermonthica* including push pull technology which involves establishing *Desmodium uncinatum* plants in the understory of the maize which are allelopathic to *S. hermonthica* (Khan et al., 2008), herbicide coated resistant maize (IR maize) (Kanampiu et al., 2002) and breeding of tolerant and resistant varieties of maize (Kamara et al., 2012). These technologies have shown to be effective under on-farm trial conditions; however their widespread adoption and uptake has been limited. Reasons for slow uptake are a complex mix of social and economic factors that influence the decisions of risk averse small-scale subsistent farmers.

In the 1990s interest in finding a disease of *S. hermonthica* for use as a potential biological control was investigated. In North Ghana *Fusarium oxysporum* isolate Foxy 2 was isolated from a diseased *S. hermonthica* plant (Abbasher et al., 1995) and a new forma specialis named *F. oxysporum* f. sp. *strigae*, which causes *Fusarium* wilt of *Striga* species was identified (Elzein et al., 2008). In 2009, the same isolate was imported into Kenya and has been subsequently tested against *S. hermonthica.* Foxy 2 was shown not to control *S. hermonthica* in Western Kenya (Avedi et al., 2014). As part of the efforts of developing a possible biological control in Kenya, diseased *S. hermonthica* plants were selected from a maize field and the pathogens were isolated and subsequently cultured which included the isolate FK3. In order to confirm whether the selected Kenya *F. oxypsorum* isolate FK3 had potential as a biological control agent of *S. hermonthica* in Kenya, our hypothesis is that pathogen, isolate FK3 taken from infected *S. hermonthica* plants in Kenya, reduces the presence of *S hermonthica* when applied to the planting hole and secondly, the use of FK3 improves the growth and yield of maize in *S. hermonthica* infested soil.

MATERIALS AND METHODS

Fungal isolate FK3

Diseased *S. hermonthica* plants were selected in a maize field in Western Kenya. Based on the speed of growth on potato dextrose agar (PDA) plates an isolate FK3 was selected as a fast growing isolate. Subsequently, this isolate was confirmed as a *F. oxysporum* (Wainwright and Viljoen, 2014). The production of FK3 was done by inoculating sterilised rice grains in plastic bags and allowing to grow for 7 days at 22°C, the rice was then removed from the bags and dried to a moisture content of approximately 5%. The dried rice was then ground to a coarse powder prior to use. Each gram contained 1.5×10^7 colony forming units (CFUs).

Pot trial protocols

Two pot experiments were undertaken during 2013 and 2014 at KARI/CIMMYT collaborative site in Kibos, Kisumu, Kenya. Plants were grown in a shade house. Each experiment consisted of a factorial design with two maize varieties and four rates of *F. oxysporum* isolate FK3. Five litres pots containing un-sterilized field soil were used with 20 pots per treatment. To each pot, 5 g of Di ammonium phosphate fertiliser (18-0-46) and a teaspoon of *S. hermonthica* seed and sand mix (approximately 1000 seeds) were mixed to the upper 5 cm layer of pot soil. Three maize seeds were sown per pot and thinned to a single seedling after germination. In both experiments, the rates of FK3 were the same; these being 7.5×10^7 (low rate), 1.5×10^8 (medium rate) and 6×10^8 (high rate) of CFUs per pot. Top dressing with CAN fertiliser was done at 6 weeks after sowing at a rate of 10 g per pot (27:0:0) (DEFRA, 2010).

Maize varieties

In the first pot trial the two varieties of maize sown were WH507, a susceptible hybrid variety from Western Seed Company Ltd, and a local farmer saved white seeded maize variety locally known as Rachar. Both varieties are short season varieties (3 month). In the second pot trail, the two varieties of hybrid maize sown were WH507 and WH403, both considered susceptible varieties from Western Seed Company Ltd.

Data collection and analysis

Parameters assessed to validate the effect of *F. oxysporum* were *S. hermonthica* plant numbers per pot at week 14 and maize plant height and leaf number. In the second pot trial, additional parameters assessed were total weight of harvested cobs in each treatment, stover weight, cob length, cob diameter, 100 seeds grain weight and fresh root weight. Data obtained was subjected to analysis of variance and significant differences determined between the means by Fishers protected LSD at $p<0.05$, using GenStat software. The interaction of two-way analysis wase not presented as none were significant.

RESULTS

The first pot trial showed that there was a significant difference in *S. hermonthica* plant numbers between the untreated pots and those treated at any rate with FK3 isolate of *F. oxysporum*, however there were no differences in *S. hermonthica* number with different rates of FK3. There were no differences in plant height or leaf number with different rates of FK3. There were no differences in *S. hermonthica* numbers for each variety; however variety WH507 gave short plants and more

Table 1. Mean number of Striga plants per pot and growth of maize (height and leaf number) in the first season pot trial.

Treatments	Striga plant number per pot (Week14)	Maize plant height (Week 11) (cm)	Maize leaf number (Week 11)
Rate of FK3			
Untreated	10.73[a]	159.2[a]	17.05[a]
FK3 lower rate	5.55[b]	162.7[a]	17.01[a]
FK3 mid-rate	5.03[b]	164.9[a]	17.18[a]
FK3 high rate	3.30[b]	163.7[a]	17.12[a]
P value	<.001	0.732	0.075
S.E	1.526	5.26	0.2252
Variety			
WH507	6.36[a]	144.5[a]	18.20[b]
Rachar	5.94[a]	180.8[b]	15.94[a]
P value	0.694	<.001	<.001
S.E	1.079	3.72	0.2372

Means in the same column and the same variable (FK3 rate or variety) followed by the same letter are not statistically different from each other.

Figure 1. The effect of applying four rates of *F. oxysporum* f. sp. *strigae* isolate FK3 at the time of maize planting (variety WH507) on the emergence of Striga plants. From left to right, Control (untreated); Low rate; Medium rate and High rate.

leaves than Rachar irrespective of treatment (Table 1).

The second pot trial showed that there was a significant difference in *S. hermonthica* plant numbers between the untreated pot soil and those treated at any rate with FK3 isolate of *F. oxysporum*, and also that the lower rate FK3 treated had higher number of *S. hermonthica* plants than the middle and higher rates of FK3. Single pot examples of the different treatments of variety WH507 showing the different Striga numbers are shown in Figure 1. There were no differences in plant height or leaf number with different rates of FK3. There was significant reduction in internode length in the untreated when compared to the medium rate FK3. Fresh root mass gave significant differences with the untreated giving the least root mass and the highest rate of FK3 gave the highest root mass. Stover weight showed that there was a significant difference between the untreated pot soil and those treated at any rate with FK3. For varieties, WH403 had

Table 2. Mean number of Striga plants per pot and growth of maize (height, leaf number, internode length, fresh toot mass and stover weight) in the second season pot trial.

Parameter	Striga plant number per pot (Week14)	Maize plant height (Week 11) (cm)	Maize leaf number (Week 11)	Maize internode length (Week 11) (cm)	Maize Fresh root mass (g)	Stover weight (kg)
Rate of FK3						
Control	21.274[c]	195.3[a]	16.58[a]	13.27[a]	182.5[a]	1.695[a]
FK3 lower rate	7.881[b]	205.4[a]	16.63[a]	14.34[ab]	209.4[b]	2.074[b]
FK3 medium rate	4.274[a]	206.1[a]	16.70[a]	14.80[b]	188.1[ab]	2.083[b]
Fk3 higher rate	3.996[a]	204.8[a]	16.46[a]	14.32[ab]	261.2[c]	2.196[b]
P value	< 0.001	0.119	0.811	0.043	0.002	0.047
S.E	2.032	5.843	0.2369	0.5741	22.11	0.2094
Variety						
WH 403	11.480[b]	197.6[a]	16.27[a]	13.84[a]	152.5[a]	1.852[a]
WH 507	7.507[a]	207.8[b]	16.89[b]	14.50[a]	268.1[b]	2.171[a]
P value	0.006	0.009	< 0.001	0.125	<.001	0.061
S.E	1.412	4.071	0.1634	0.3990	15.64	0.1223

Means in the same column and the same variable (FK3 rate or variety) followed by the same letter are not statistically different from each other.

significantly more *S. hermonthica* plants than WH507, but WH403 had significantly shorter plants, fewer leaf number and less root mass than WH507 (Table 2).

The maize yield parameters of the second pot trial gave significantly higher maize seed weight (100 grains) and total cob weight using the higher rate of FK3 when compared to the untreated control. However there were no significant differences in the cob diameter, length or moisture content with any rate of FK3. The variety WH403 gave significantly lower grain weight and higher total cob weight than WH507 but no other differences between cob diameter, length or moisture content (Table 3).

DISCUSSION

The two trials consistently show that the *F. oxysporum* isolate FK3 reduced the number of *S. hermonthica* plants when maize of different varieties was grown in pots. The reduction of *S. hermonthica* was greatest with the two highest rates of FK3 in trial 2. The use of the isolate Foxy 2 in Benin and Burkina Faso showed that *S. hermonthica* numbers were reduced in maize and sorghum crops (Venne et al., 2009), however when used in Kenya Foxy 2 had no effect on S. hermonthica reduction (Avedi et al., 2014). A range of *S. hermonthica* pathogens with visible characteristics of *F. oxysporum* was sampled from maize fields in Western Kenya and West Africa in 2012. These pathogens were isolated and comparative analysis undertaken showed that the populations from the two regions, Kenya and West Africa, were genetically different from each other (Wainwright and Viljoen, 2014). *S. hermonthica* is present in both East and West Africa; however a recent genetic study with microsatellite markers showed that a small portion (8%) of the variation occurred between regions of origin of the populations (Bozkurt et al., 2015). The genetic variation in both *S. hermonthica* and *F. oxysporum* pathogens may explain the regional specificity of *F. oxysporum* isolates as a potential biological control agents.

The preparation of the FK3 isolate for soil Inoculation was based on rice media that was dried and coarsely ground prior to application to the planting hole. This method of preparation showed considerable promise as a simple low cost method of manufacturing the inoculum and then treating the soil. This is similar to the concept of the Pesta granule as a means of inoculating the soil (Elzein and Kroschel, 2006), however the preparation based on rice grains is simpler than the Pesta granule that contained semolina, kaolin, and sucrose as well as the fungal inoculants. The efficacy of soil applied biological control agents

Table 3. Mean maize yield parameter of maize (grain weight, cob diameter, cob length, seed moisture content and total cob weight per plant) in the second season pot trial.

Treatments	Grain weight (100 seeds) (g)	Cob diameter (cm)	Cob length (cm)	Seed moisture content (%)	Total cob weight (kg)
Rate of FK3					
Control	28.50[a]	3.00[a]	10.65[a]	26.80[a]	1.689[a]
FK3 lower rate	29.00[ab]	3.10[a]	11.20[a]	29.80[a]	1.797[ab]
FK3 medium rate	28.70[a]	3.10[a]	10.90[a]	27.45[a]	1.776[ab]
FK3 high rate	30.00[b]	3.15[a]	10.95[a]	30.70[a]	1.995[b]
P value	< 0.001	0.816	0.056	0.624	0. 031
S.E	1.637	0.1229	0.3432	1.112	1.562
Variety					
WH 403	24.75[a]	3.08[a]	11.45[a]	28.33[a]	2.072[a]
WH 507	33.25[b]	3.10[a]	10.40[a]	29.05[a]	1.556[b]
P<0.05	0.026	0.57	0.063	0.42	0.038
S.E	1.223	0.0869	0.2760	1.047	1.353

Means in the same column and the same variable (FK3 rate or variety) followed by the same letter are not statistically different from each other.

such as FK3 appears to rely on dose, as increasing the rate showed a decrease in *S. hermonthica* plant numbers. However there was no interaction between dose rate and variety on *Striga* numbers. There are practical and economic limitations on how much inoculum will be able to be applied to the field situation and further evaluation of this aspect will need to be assessed before such an application method may be applied in the field.

The performance of maize showed there were indications that when exposed to *S. hermonthica*, growth parameters were improved with the use of FK3 such as internode length, fresh root mass and stover weight. Similarly harvested yield of maize showed increases in grain and cob weight. However pot trials are not a reliable enough method to evaluate growth and yield studies based on twenty plants per treatment. However the pot trials results reported here strongly suggest the need to move to open field trials to assess the impact of FK3 on maize yield when stressed by S. hermonthica infestations. The maize variety WH403 produced significantly more *S. hermonthica* plants than WH507, whilst there were no differences between WH507 and the local variety Rachar. Therefore, variety susceptibility with *S. hermonthica* is important, but the response to FK3 in *Striga* number reduction was evident in all varieties which indicates the wider applicability of this technique to a range of germplasm. The level of *S. hermonthica* in the first trial for WH507 (6.36 plants per pot) were very similar to those in the second trial (7.5 plants per pot) for the same variety which demonstrates the consistency of the methodology used.

The use of biological control treatment for the reduction of *S hermonthica* has numerous benefits for the control or reduction *S. hermonthica*. The inoculum can be used on both hybrid and farmer saved seed, the latter being of continuing importance as a farming practice in many sub-Saharan African regions. The biocontrol has the potential to be used on a range of species that are affected by *S. hermonthica* such as sorghum, rice and millet as well as maize though this needs verification. However this work in conjunction with other findings suggests that *S. hermonthica* pathogens are region specific.

Conflict of interests

The authors have not declared any conflict of interest.

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