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Biocontrol agent *Fusarium oxysporum* f.sp. *strigae* has no adverse effect on indigenous total fungal communities and specific AMF taxa in contrasting maize rhizospheres

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ABSTRACT

We studied the effects of *Fusarium oxysporum* f.sp. *strigae* (Fos), a soil-borne biocontrol agent (BCA) against *Striga hermonthica*, on total fungal and arbuscular mycorrhizal fungal (AMF) taxa in rhizospheres of maize in both clayey and sandy soil. Effects of Fos-BCA 'Foxy-2' were evaluated against (1) *S. hermonthica* presence, and (2) organic fertilization with *Tithonia diversifolia* residues at 14, 28 and 42 d after 'Foxy-2' inoculation, via DNA-based quantitative PCR and TRFLP fingerprinting. In both soils, 'Foxy-2' occasionally promoted total fungal abundance, while the community composition was mainly altered by *T. diversifolia* and *S. hermonthica*. Notably, 'Foxy-2' stimulated AMF *Gigaspora margarita* abundance, while *G. margarita* was suppressed by *S. hermonthica*. Total fungal and AMF abundance were promoted by *T. diversifolia* residues. In conclusion, 'Foxy-2' resulted in no adverse effects on indigenous rhizosphere fungal communities substantiating its environmental safety as BCA against *S. hermonthica*.

1. Introduction

The parasitic weed *Striga hermonthica* is a major constraint to cereal production in Sub-Saharan Africa causing yield losses worth US$ 9 billion (Ejeta, 2007; Gibbon et al., 2007). *S. hermonthica* parasitizes staples such as millet (*Pennisetum americanum*), sorghum (*Sorghum bicolor*), maize (*Zea mays*), and rice (*Oryza sativa*) (Elzein and Kroschel, 2004; Marley et al., 2004). It infests more than 50 million hectares of farmland with intensifying dissemination in Sub-Saharan Africa, which makes it one of the gravest threats to food security in this region (Parker, 2012).

Control of *S. hermonthica* remains challenging due to its very high seed production per plant, with seed survival rates in soils of more than ten years (Parker and Riches, 1993; Van Mourik, 2007). It has been widely accepted that a single control method is not effective against *S. hermonthica*, hence, integrated approaches are postulated as control strategies (Menkir and Kling, 2007; Hearne, 2009; Atera et al., 2012).

The combination of biological control agents (BCAs) such as *Fusarium oxysporum* f.sp. *strigae* (Fos) along with tolerant crop varieties provided respectable control against *S. hermonthica* under field conditions in Burkina Faso, Benin and Nigeria (Schaub et al., 2006; Venne et al., 2009). In particular, the Fos strain 'Foxy-2' was effective in suppressing all developmental stages of *S. hermonthica* ranging from germination to flowering (Elzein and Kroschel, 2004; Ndambi et al., 2011). In addition, Ndambi et al. (2011) reported that 'Foxy-2' colonized endophytically the roots of the host crop (e.g., sorghum), where the biocontrol activity of 'Foxy-2' was initialized after *S. hermonthica* attacked the root system.

In contrast to previous studies performed in West Africa (e.g., Schaub et al., 2006; Venne et al., 2009), recent efficacy studies of 'Foxy-2' in Kenya showed no effective biocontrol ability of 'Foxy-2' against *S. hermonthica* (Avedi et al., 2014). These contradictory results were explained by potential genetic distinctions between Eastern and Western African *S. hermonthica* varieties, but also by abiotic and biotic environmental factors influencing the proliferation and hence efficacy of 'Foxy-2' in foreign ecosystems. Zimmermann et al. (2015), using a Fos specific and quantitative monitoring tool, followed the fate of BCA Fos after inoculation into

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foreign soil ecosystems, and showed that Fos proliferation was controlled by physico-chemical soil characteristics and by the availability of organic resources, for which Fos is in competition with indigenous microorganisms in the rhizosphere of the host crop. The latter fact requires particular attention as Fos is a soil borne fungus and proliferates saprotrophically and endophytically in crop rhizospheres and roots, respectively (Ndambi et al., 2011).

Soil microorganisms maintain critical soil functions including nutrient cycling as well as turnover and stabilization of soil organic matter (van der Heijden et al., 2008; Kunlanit et al., 2014). A range of soil microorganisms have been shown to suppress soil-borne plant diseases and to promote plant growth (Compant et al., 2005; Rasche et al., 2006a,b; Liu et al., 2007). With respect to resource acquisition in soils, it was recently speculated that there might exist a potential resource competition between Fos and indigenous soil microorganisms (Zimmermann et al., 2015). Hence, it could be hypothesized that the release of Fos in soils may have a considerable effect on the abundance and community composition of functionally relevant indigenous soil microorganisms which may in turn influence crop health and yield. The impact of the Fos strain 'Foxy-2' on the abundance of total indigenous bacterial communities and plant-beneficial prokaryotic nitrifiers in a maize rhizosphere was emphasized by Musyoki et al. (2015) who detected no negative side effects of 'Foxy-2' on root-associated bacteria.

In the study we present here, we focused on community dynamics of rhizosphere fungi as these may colonize similar niches as Fos and thus compete for similar resources in the rhizosphere (Winding et al., 2004). We put major emphasis on functionally relevant members of the fungal community focusing primarily on arbuscular mycorrhizal fungi (AMF) colonizing crop roots. The focus on AMF is justified due to their beneficial effects on crop growth and crop stress compensation (Smith and Smith, 2012). We studied the response of fungal communities to Fos inoculation in two contrasting (clayey Humic Nitisol versus sandy Ferric Alisol) soils from Kenya which were not naturally infested with Fos. A rhizobox experiment was conducted in which the selected soils were treated with the Fos strain 'Foxy-2' via seed coating of a tropical maize variety used as a test crop. Two additional factors were considered: (1) presence of *S. hermonthica*, and (2) application of *Tithonia diversifolia* residues, a widely used green manure in Sub-Saharan Africa (Gachengo et al., 1998; Jama et al., 2000; Opala et al., 2015), to cover the hypothesized resource competition effects. *T. diversifolia* is classified as high quality organic fertilizer with low C/N ratio (Chivenge et al., 2009) and provides an easily accessible C source and high N availability to stimulate indigenous fungal communities (Zimmermann et al., 2015). The response of the total fungal abundance was monitored at 14, 28 and 42 d after inoculation (DAI) using DNA-based quantitative polymerase chain reaction (qPCR), while fungal community composition (terminal restriction fragment length polymorphism (TRFLP) fingerprinting) and AMF taxa abundance (qPCR) were monitored at 42 DAI.

2. Material and methods

2.1. Rhizobox experiment

2.1.1. Preparatory work

The model Fos isolate 'Foxy-2' was obtained from *S. hermonthica* collected from North Ghana (Abbasher et al., 1995). Taxonomic identification of the isolate was confirmed by Julius-Kühn-Institut (JKI), Berlin, Germany, where it is deposited under accession number BBA-67547-Ghana. Maize (*Z. mays* variety 'WH507', provided by Western Seed Company Ltd., Kitale, Kenya) was used as a test crop. The selected variety is highly preferred by smallholder farmers in Western Kenya due to its tolerance to *S. hermonthica*. Maize seeds were coated with dried 'Foxy-2' chlamydospore inoculum (1.15×10^5 colony forming units per seed) homogenized into 20% arabic gum used as adhesive through a special seed treatment technology (Elzein et al., 2006; seed coating processed by SUET GmbH, Eschwege, Germany) to provide uniform inoculum coverage. *S. hermonthica* seeds (originating from Sudan) were surface sterilized according to Elzein et al. (2010) and germination viability of seeds (75%) was checked as described by Kroschel (2002).

2.1.2. Rhizobox set-up

Rhizoboxes ($3 \times 7 \times 20$ cm) were filled with dry soils (165 g) derived from two contrasting field sites in the central highlands of Kenya: Embu (0° 30′ S, 37° 30′ E; 1380 m above sea level (a.s.l.)) and Machanga (0° 47′ S, 37° 40′ E; 1022 m a.s.l.). Soils differed greatly in physical properties: the Embu soil was a clayey Humic Nitisol (17% sand, 18% silt, 65% clay) derived from basic volcanic rocks, while the Machanga soil was a sandy Ferric Alisol (66% sand, 11% silt, 22% clay) derived from granitic gneisses (IUSS Working Group WRB, 2015). Each rhizobox was filled at the bottom with a 1 cm ground layer of vermiculite (grain size 3–8 mm) for drainage improvement. On top of this layer, soil adjusted to 50% water holding capacity was added.

Both soils were infected artificially with disinfected *S. hermonthica* seeds (20 mg seeds 165 g dry soil^{-1}). *S. hermonthica* seeds were thoroughly mixed with the moist soils and preconditioned at 28 °C in the dark for 7 d (Kroschel, 2002). After this step, pre-germinated maize seedlings were introduced into the rhizoboxes. After planting of seedlings, a 1 cm layer of vermiculite was placed as the top layer to reduce evaporation.

Boxes were placed in an incubation chamber (12 h with artificial light (1000 μmol m^{-2} s^{-1}) and 12 h darkness at 28/21 °C (day/night) for 6 weeks). Two and 4 weeks after the start of incubation, soil was fertilized with inorganic liquid fertilizer (4 ml each rhizobox with 0.2% Wuxal N-P-K (8-8-6), Aglukon GmbH, Düsseldorf, Germany) to avoid nutrient deficiency. In addition, a treatment with organic residues was included by incorporating air-dried and ground (particle size 1–3 mm) leaf and stem material of *T. diversifolia* (1 g dry matter 100 g dry soil^{-1}) into soils before planting of maize seedlings. Non-fertilized treatments were included as controls.

The rhizobox experiment was arranged as a completely randomized design with 6 treatments with 3 replicates for each soil type: (i) uncoated maize seeds with no *S. hermonthica* (C), (ii) uncoated maize seeds and *S. hermonthica* (C + S), (iii) coated maize seeds with 'Foxy-2' (F), (iv) coated maize seeds with 'Foxy-2' and *S. hermonthica* (F + S), (v) coated maize seeds with 'Foxy-2' and *T. diversifolia* (F + T), and (vi) coated maize seeds with 'Foxy-2', *S. hermonthica* and *T. diversifolia* (F + S + T).

2.1.3. Rhizosphere and bulk soil samplings

Rhizosphere samples for molecular analyses were taken 14, 28 and 42 d after inoculation (DAI). For this step, the rhizobox was opened and approximately 2 g of root adhered soil was taken carefully from several positions in order not to damage the root system. Rhizosphere soil was gently scraped off with sterile forceps and transferred into sterile sampling bags. Soil samples (bulk soil) for chemical analyses were obtained at 42 DAI. Rhizosphere soil samples were freeze dried and stored at −20 °C until molecular analysis, while bulk soils for chemical analyses were directly maintained at −20 °C. One proportion of the obtained rhizosphere soil samples was used to study the impact of 'Foxy-2' on indigenous prokaryotic communities (Musyoki et al., 2015) while another was used in the present study to assess the impact of 'Foxy-2' on indigenous fungal communities.