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Cytological investigations on colonization of sorghum roots by the mycoherbicide Fusarium oxysporum f. sp. strigae and its implications for Striga control using a seed treatment delivery system

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1. Introduction

The root parasitic weed Striga hermonthica (Del.) Benth. is one of the major threats to cereal production in the African savannas, directly marginalising capacity for food production by sometimes causing total crop loss in farmers' fields particularly under drought conditions and infertile soils (Bebawi and Farah, 1981; Sauerborn, 1991; Parker and Riches, 1993; Haussmann et al., 2000; Marley et al., 2002; Gressel et al., 2004; Ejeta, 2007). The significant yield reductions caused by Striga in staple cereals like sorghum (Sorghum bicolor L. Moench), maize (Zea mays L.), millet (Pennisetum glaucum L.) and upland rice (Oryza sativa L.) aggravate hunger and poverty for millions of subsistence farmers in Africa. The parasite has a complex life cycle, which is intimately linked to that of its host plants. Striga seed germination occurs only when mature seeds are preconditioned by exposure to warm moist conditions for several days followed by germination stimulants derived from host and some non-hosts roots (Worsham, 1987). A germ tube growing close to the host roots elongates towards the roots, and upon contact develops a haustorium, an organ of attachment to the xylem vessels of the host. The haustorium is the organ of acquisition of nutrients and water from the host (Rich and Ejeta, 2007), causing drought stress and wilting of the host. After several weeks of underground development the parasite emerges above the soil surface and starts flowering and subsequent seed production. Up to 100,000 seeds can be produced by a single plant, which can

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Abstract

The application of the potential Striga-mycocidal fungus Fusarium oxysporum f. sp. strigae (Foxy 2) by seed coating is an appropriate option for delivering and establishing the biocontrol agent in the rhizosphere, the infection zone of the root parasitic weed Striga hermonthica. Cytological investigations using light and transmission electron microscopy were performed to assess the pattern and extent of colonization of sorghum roots and shoots by Foxy 2, applied as film-coat on seeds. Germination of Foxy 2-treated seeds was similar to un-treated plants. During sorghum root development, mycelia of Foxy 2 started from seed coat colonizing root surfaces. The intensity of root colonization increased with time. However, hyphae were not found in the apical zone of the roots. Hyphae growing on the surface of the roots were observed penetrating rhizodermal cells including root hairs, and colonizing the intercellular space and the cells of the cortical parenchyma. Even after four weeks, hyphae were not penetrating the endodermal layer to invade the tissues of the central cylinder. In addition, hyphae of Foxy 2 were also completely absent in the xylem vessels in sorghum shoots even after 11 weeks of sowing, which further proves the non-pathogenicity of the fungus to sorghum. Foxy 2 showed high efficacy in controlling Striga in combination with both tolerant and susceptible sorghum varieties in a root chamber experiment, as reflected by the high percentage of diseased Striga seedlings (95% and 86%, respectively), recorded after 26 days of sowing. The intensity of fungal root colonization, which coincides with the peak of Striga attachments to its host root, will ensure high fungal efficacy and will facilitate the practical use of Foxy 2 against Striga using seed treatment delivery. Indeed, this proof of sorghum non-pathogenicity of the fungus will encourage the acceptance of Foxy 2 by authorities and farmers in Africa.

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remain viable for up to 15 years and lead to a re-infestation of the field (Parker and Riches, 1993). Thus, if host plants are frequently cultivated, the Striga seed population in the soil increases tremendously and cropping of host plants becomes less and less economically feasible. Hence, a biocontrol agent that is able to attack all developmental stages of Striga including seeds is necessary for causing sustainable depletion of the weed seed bank.

In recent years, extensive research efforts and promising results have been made on biological control of Striga hermonthica using the soil-borne fungus Fusarium oxysporum as a mycoherbicide (Abbasher et al., 1995; Ciotola et al., 1995; Kroeschel et al., 1996; Marley et al., 1999). Hence, the potential of Striga biocontrol technology as an excellent integrative tool to augment other tricks within integrated approach has gained considerable attention. The Striga-mycoherbicidal strain F. oxysporum Schlecht f. sp. strigae Elzein et Thines abbreviated as “Foxy 2”, is a promising mycoherbicidal candidate against S. hermonthica. The isolate is highly virulent and host specific to the genus Striga (Kroeschel et al., 1996; Elzein and Kroeschel, 2006a). It possesses a unique DNA sequence enabling it to be considered as a new forma specialis (f. sp. striae) (Elzein et al., 2008). Proper formulation, delivery and timely establishment of the potential biocontrol agent “Foxy 2” in the infection zone of Striga is necessary for ensuring a high biocontrol efficacy. Hence, progressive research aiming at facilitating and enhancing field application and integration of Foxy 2, resulted in an optimized inoculum production technique of the isolate based on inexpensive agricultural by-products (Elzein and Kroeschel, 2004), and in the development of Foxy 2 into Pesta granular formulations or delivery systems using seed treatment technology (Elzein and Kroeschel, 2006b; Elzein et al., 2006). The advantage of delivering Striga-mycoherbicides by seed treatment procedure over Pesta formulation is that it requires significantly less inoculum amount, establishes the antagonist at the infection sites of Striga, and provides a simple, economic, and easy delivery system of biocontrol for subsistence farmers in Sub-Saharan Africa (Ciotola et al., 2000; Elzein et al., 2006).

For controlling root and soil-borne diseases by seed treatment, the antagonists must be able to grow from the seed and colonize the developing roots (Lifshitz et al., 1986; Elad and Chet, 1987; Callan et al., 1990; Jenson and Wolfhezel, 1992). Using a delivery system, where sorghum seeds were coated with Foxy 2 inoculum using gum arabic as an adhesive, a promising control efficacy of Striga was achieved in preliminary investigations (Elzein et al., 2006), which suggests the ability of the fungus to colonize the root system of the sorghum plant. Previous microscope observations revealed differences in root colonization intensity of pathogenic and non-pathogenic strains of F. oxysporum on different target crops (Alabouvette et al., 2001; Bao and Lazarovits, 2001; Benhamou and Garand, 2001; Olivain et al., 2003). In these studies, root inoculation was done either by direct dipping the root system into a conidial suspension or transplanted in soil heavily infested with conidia. However, in our current research we use a different strain and a novel delivery approach (seed treatment) for microbial agents for root parasitic weeds where the fungal mycoherbicides were film-coated on cereals seeds. Taking into consideration the intimate interaction between host and parasite, time and extent of colonization of the host roots by the fungus is utmost important for ensuring a significant parasite biocontrol and for providing additional evidence supporting the non-pathogenic behaviour of Foxy 2 as a biocontrol agent on non-targets.

The objectives of this study were to perform cytological investigations using transmission electron and light microscopy to study the pattern and intensity of colonization of sorghum roots by Foxy 2 as non-pathogenic strain. Efficacy and implication of Foxy 2-colonized roots after seed treatment inoculation for successful biocontrol of Striga was also evaluated in combination with Striga-tolerant and susceptible sorghum cultivars, aiming at providing a potential integrated Striga management package.

2. Material and methods

2.1. Fungal strain

The fungal strain F. oxysporum f. sp. strigae “Foxy 2” used in this study was isolated from severely diseased S. hermonthica collected in North Ghana by Abbasher et al. (1995). Taxonomic identification of the isolate was confirmed by the Federal Biological Research Center for Agriculture and Forestry, Berlin, Germany, where the isolate was deposited under accession number BBA-67547-Ghana. Since then the isolate was preserved on Special Nutrient poor Agar (SNA)-medium (Nirenberg, 1976) with 5% (v/v) glycerol at −40 °C in the Institute of Plant Production and Agroecology in the Tropics and Subtropics, University of Hohenheim, Stuttgart, Germany.

2.2. Inoculum preparation and seed coating procedure

As fungal inoculum, dried chlamydospores were prepared by cultivating actively growing colonies of Foxy 2 in a medium containing 0.5% w/v maize stover (<500 μm) and 20% v/v of wheat (Triticum aestivum L.)-based stillage (the spent fermentation broth of ethanol production with poor nutrient constituents) in deionized water. The procedure was previously described (Elzein and Kroeschel, 2004). To prepare dried chlamydospore inoculum, yield of fresh chlamydospores in the pellet was collected after centrifugation and air-dried at room temperature. Thereafter, dried chlamydospores were ground to a fine powder (<100-μm) to facilitate the process of coating and to ensure an even distribution of fungal inoculum on seed films. Viability of the dried material, determined by plating serial dilutions on potato dextrose agar (PDA) plates (Sigma GmbH, Steinheim, Germany), and incubating at room temperature (22 ± 3 °C) for three days, was 1.0 × 10⁷ CFU g⁻¹ (colony forming unit).

The seeds of two sorghum varieties [the Striga-susceptible variety “Cowbuala” from Burkina Faso was provided by the International Institute of Tropical Agriculture (IITA-Benin Station), and the Striga-tolerant variety “Wad Admed” was provided by the Agricultural Research Corporation (ARC), Sudan] were used in this study. The seeds were first surface sterilized by complete immersion in 1% sodium hypochlorite (NaOCl) for 5 min. Then they were rinsed with tap water until water was clear to ensure complete removal of NaOCl, and dried overnight at room temperature. Thereafter, all sorghum seeds were film-coated with a homogenized suspension of gum arabic (AG, 40%) and dried chlamydospores of Foxy 2 (Elzein et al., 2006) using a special seed treatment technology of SUET (SUET Saat-u. Ernte-Technik GmbH, Eschwege, Germany). For coating 1 g of sorghum seeds, 0.048 g of fungal dried chlamydospores with 1.0 × 10⁷ CFU g⁻¹ were required. The coated seeds were not only uniformly coated, but also abrasion- and dust-free, and with excellent fluidization for assuring high quality coated seeds. All seeds were stored in sealed transparent polyethylene plastic bags (120 × 90 mm, 50 μm, Clausen Papier GmbH, Stampe, Germany) at 4 °C before used for further tests.

The viability of Foxy 2 on coated sorghum seeds was assayed by dissolving the films of three coated seeds (0.1 g) in 5 ml deionized water in a glass test tube. After serial dilution, 0.1 ml was planted on PDA and CFUs per seed were counted after three days of incubation at 25 °C. Three replicates per treatment of three replicate plates were used (i.e., nine plates per treatment). The variety “Wad Ahmed” contained 2.4 × 10⁶ CFUs per seed, while “Cowbuala” had 2.7 × 10⁶ CFUs per seed.
2.3. Cytological investigations

2.3.1. Plant material and cultivation

For all cytological investigations the Sorghum variety “Wad Admed” were used to study the pattern and extent of colonization of sorghum roots and shoots by Foxy 2 hyphae. Plants were grown in vitro between sterile filter paper for 3 weeks, and for longer periods in pots with sterile soil. For in vitro cultivation five coated or uncoated (control) sorghum seeds were arranged in rows between twolayers of sterile filter paper (Whatman GF/A, 30 × 20 cm), moistened with sterile water, rolled and wrapped in sterile transparent plastic bags (International Seed Testing Association (ISTA), 1993). Then the bags were incubated in an upright position at 25 °C in darkness for 48 h and thereafter in 12/12 h light/darkness (Elzein et al., 2006). From each treatment the roots of 4 fungal-treated sorghum plants and 4 of the corresponding controls were collected. Sorghum root samples (0.5–1.0 cm in length each) were collected 1, 2, and 3 weeks after germination from the upper, middle and bottom part of each sampled root for microscopic examinations.

For longer (>3 weeks) periods of cultivation, five Foxy 2-coated sorghum 2-seeded sorghum seeds were sown in each plastic pot (18 × 18 × 18 cm) filled with 4 kg of garden soil (pH 6.7, 5.4% OM), steam-sterilized for 8 h before it was used in pot experiment (Elzein et al., 2006). Pots containing uncoated seeds were set up as controls. Two weeks after sowing, the plants were thinned to three plants per pot, and were watered when necessary. All treatments including controls were replicated four times and arranged as a completely randomized design. The experiment was carried out under glasshouse conditions, where the temperature was adjusted at 34 ± 1 °C/24 ± 1.5 °C day/night, and relative humidity ranged from 36 to 71%. Lighting was natural except when artificial lights from HQL-Lamps (1000 W) were used to supplement natural light and to extend day length to 12 h when necessary. The plants were fertilized with 200 ml of a liquid fertilizer, 0.2% Wuxal® N-P-K (8-6-8) (AGLUKON, GmbH Düsseldorf, Germany), which were split-applied as 100 ml per pot, when the plants were 2 and 4 weeks old. For examining sorghum root colonization, root sections of 0.5–1.0 cm in length each, were taken from top, bottom and tip of the 22-cm in length roots, collected at 4 weeks after planting (WAP). In addition, pieces of sorghum shoots (0.5–1.0 cm) were also taken from bottom, middle, and top of the main stems at 5, 6 and 11 WAP for testing sorghum shoot colonization by Foxy 2.

2.3.2. Light- and transmission electron microscopy

2.3.2.1. Light microscopy (LM). Foxy 2-treated and un-treated root samples from sorghum plants, 1, 2 and 3 weeks after germination and grown between sterile filter paper as well as 4-week old root samples from plants grown in sterile soil were collected. Only roots grown in soil were thoroughly washed before running tap water to remove all adhering particles. Then small samples of the upper region of the roots near the seed, 0.5 × 1–2 mm2 in size, were cut with a razor blade and immediately fixed in 2.5% (v/v) buffered glutaraldehyde (0.1 M phosphate buffer, pH 7.2) for one hour and postfixed in 1% (w/v) buffered osmium tetroxide (0.1 M phosphate buffer, pH 7.2) for an other hour. The samples were rinsed three times in distilled water, dehydrated in a series of acetone solutions [30, 50, 70, 100, 100, 100, 100, (v/v)], one hour each step, infiltrated and embedded in Epon 812 substitute (Agar 100 resin, Plano) and polymerized in flat moulds at +60 °C. Ultrathin sections were obtained with a diamond knife and the Ultracut UCT (Leica) microtome. They were collected on Pioloform (Science Service, Munich) and carbon coated copper grids, stained with uranyl acetate followed by lead citrate (Reynolds, 1963), and examined with a transmission electron microscope (EM 10, Zeiss) at 60 kV. TEM negatives were scanned (Epson Perfection 2450 Photo) and imported in Photoshop, where brightness and contrast were adjusted.

2.4. Bioassay for efficacy evaluation

Following the cytological investigations, the efficacy of 1-year stored Foxy 2-coated sorghum seeds of Striga-tolerant Wad Ahmed and susceptible Cowbaula varieties (with 70% fungal viability recovery per seed) in controlling S. hermonthica was evaluated using root chamber experiments described by (Linke et al., 2001). Striga hermonthica seeds (50 mg) from Sudan (germination 95%), surface sterilized with NaOCl, were evenly distributed on moistened strips of 26 × 6 cm2 microfiber glass filter paper (microfiber glass Whatmann GF/A) and placed in the chamber (26 × 6 × 3 cm3) facing the plexiglass. The chambers were then filled with sterilized sand and wrapped in black plastic supported by rubber bands to prevent light penetration. For preconditioning of the Striga seeds, the chambers were moistened and then incubated at room temperature (22 ± 3 °C) for 7 days. Thereafter, the chambers were transferred to the glasshouse, described in Section 2.3.1, and three of either Foxy 2-coated or uncoated sorghum seeds for each treatment were sown in each chamber between the filter paper and the plexiglass. The treatments tested included: (i) Foxy 2-coated tolerant sorghum (Foxy ST), (ii) Foxy 2-coated susceptible sorghum (Foxy SS), (iii) uncoated tolerant sorghum (Control ST), and (iv) uncoated susceptible sorghum (Control SS). The treatments were arranged as a complete randomized design with four replicates. The chambers were placed at an angle (30°) in plastic containers (40 × 34 × 12 cm, length, width, depth) to enhance the growth of sorghum roots near the surface of the plexiglass. Two weeks after sowing, the plants in the chambers were fertilized with a solution containing 0.2% liquid fertilizer (Wuxal® N-P-K (8-8-6)). Efficacy of Foxy 2-coated sorghum seeds in controlling Striga...
was evaluated at 15, 21, and 26 days after sowing. At each evaluation date, root length was measured, and number of healthy and green versus brown, wilted, rotted or with necrotic lesions attached *Striga* seedlings were monitored using a binocular microscope.

For statistical analysis, all data including the arcsine-transformed percentages of diseased *Striga* plants, were tested for normal distribution and variance homogeneity with Shapiro–Wilks-W-Test and the Univariate-Test (Cochrans C, Hartley, Bartlett), respectively. Subsequently, one-way analysis of variance ANOVA was carried out using the STATISTICA software (StatSoft Inc., 2002). In addition, two-way-ANOVA was applied to verify the individual interactions (statistical differences) among the fungal mycoherbicide (as factor I) and sorghum varieties (as factor II). Significant differences between the mean values were determined by Fisher’s Least Significant Difference (LSD) at a significance level of \( p < 0.05 \). All results were presented as means of the original data with the standard error of the means, unless otherwise described.

3. Results

3.1. Colonization of Foxy 2 in sorghum roots

In general, Foxy 2-treated plants developed stronger roots than control plants in soil and between filter papers. All sorghum roots exhibited the typical anatomy of monocotyledonous plants with a...
central cylinder surrounded by an endodermis, cortical parenchyma, and rhizodermis. Within two weeks the innermost cortical layer of the roots differentiated from a characteristic primary endodermis with casparian strips (suberin lamellae) to a tertiary endodermis with a thick inner tangential cell wall. After three to four weeks many stem-borne adventitious roots developed while the first built roots were already in a state of senescence.

Germination of seeds was not negatively influenced by the seed cover containing chlamydospores of Foxy 2. Mycelia of Foxy 2 emerged from chlamydospores during root development and started to colonize root surfaces. Only few hyphae were observed on one week old root samples and there were no hyphae visible on root tips (Fig. 1a). In older plants (after two, three, and four weeks), the root surfaces were colonized by Foxy 2; the upper parts of the roots were completely covered with thick mycelia (Fig. 1b) that gradually thinned out towards the root tips. Hyphae growing on the surface of the roots were observed penetrating rhizodermal cells. After two weeks, hyphae of Foxy 2 colonized the intercellular space of the cortical parenchyma (Fig. 1c) and from there they were also able to penetrate the cortical parenchyma cells. They were found inside many cells of the cortex as well as (inside) rhizodermal cells including root hairs (Fig. 1d). There were no reactions of the host cells detectable. In the presence of Foxy 2, the cortex of the roots appeared damaged by hyphae after three to four weeks. Compared to the control only remains of the cortex cell walls or a few collapsed cell layers were left (Fig. 1e). Some of the four-week-old control roots, which were already in a state of senescence, showed some collapsed but clearly not destroyed cells (Fig. 2a) compared to the roots grown in the presence of Foxy 2 (Fig. 2b). Transmission electron micrographs revealed variable stages of degraded cell walls around hyphae as well as condensed and disintegrated cytoplasm where cell organelles were no longer distinguishable (Fig. 2c). After four weeks the central cylinder of the roots still showed intact vessels and xylem parenchyma with functional cytoplasm (Fig. 2d) while most of the cortex cells were digested by hyphae (Fig. 2b, c). The central cylinders were still en-

![Fig. 2. Light- and transmission electron micrographs of sorghum roots grown in soil after four weeks. (a) Light micrograph of a cross section of an upper part of sorghum root (control): After four weeks the tertiary endodermis (e) around the central cylinder (cc) is full developed. Rhizodermis and the cortical parenchyma (co) showing already signs of senescence as most of the cells are collapsed. (b) Light micrograph of a cross section of an upper part of sorghum root, grown in the presence of Foxy 2 after four weeks: Vast areas of the cortical parenchyma are disappeared (asterisks). Only few rows of cells and some hyphae (arrows) are left, the other cells of the cortex (co) are disintegrated by Foxy 2 and washed away before preparation. The central cylinder (cc) surrounded by the endodermis (e) is still intact. (c) Transmission electron micrograph of a cross section of an upper part of sorghum root, grown in the presence of Foxy 2 after four weeks: Detail of collapsed and destroyed cortical parenchyma cells (cp). In the cortex an active hypha (hy) of Foxy 2 is visible. The cortical parenchyma cells showing variable signs of destruction; partly degraded cell walls and degraded cytoplasm (asterisks). (d) Transmission electron micrograph of a cross section of an upper part of sorghum root, grown in the presence of Foxy 2 after four weeks: Detail of an endodermal cell (e), cortical parenchyma cell (cp), and parts of the central cylinder; xylem parenchyma cells (xp) and a vessel (v). The collapsed endodermal cell (e) is showing the typical thick inner tangential cell wall. While there are no organelles visible in the dark stained cytoplasm of the endodermal cell and the cortical parenchyma cell (cp), the central cylinder is showing an intact vessel (v) and xylem parenchyma cells with functional cytoplasm (xp).]
closed by the thick walled endodermis (Fig. 2b and d). Only the thin outer walls of the endodermal cells were collapsed (Fig. 2d). Hyphae did not penetrate the thick walled endodermis with its suberin lamellae and were never found in vessels or xylem parenchyma of the central cylinder of the root (Fig. 2d). Thus, the colonization of Foxy 2 in the cortex of the roots was not affecting the tissues of the central cylinder. Hyphae of Foxy 2 were growing in the cortex of the roots degrading cell walls and cytoplasm of the parenchymatic tissue step by step (Fig. 2c). In addition, during four weeks of colonization Foxy 2 did not develop conidia or chlamydospores in the cortical parenchyma of roots.

3.2. Non-colonization of Foxy 2 in sorghum shoots

The anatomy of sorghum shoots from seeds treated with Foxy 2 was similar to that of un-treated control. The shoots showed the characteristics of most monocotyledonous shoots with vascular bundles scattered within the ground parenchyma and being more closely packed together in the periphery almost forming a ring around the shoot. Shoots of five, six, and 11 weeks old plants did not show any great anatomical difference, only an increase in lignification of vascular bundles and hypodermal tissue occurred with increasing age. There was a complete absence of hyphae in all vessels (Fig. 3) and in all parts of the shoots and at all stages of growth.

3.3. Efficacy of Foxy 2 in controlling Striga

Efficacy of Foxy 2 in controlling S. hermonthica was evaluated based on the percentage of diseased/dead Striga seedlings in the treated root chambers compared to the controls. In general, the fungus Foxy 2 caused severe disease and death of Striga seedlings compared to the control treatments, where most of Striga seedlings were healthy and vigorous (Fig. 4a). Striga seedlings (SL) were considered diseased when they turned brown and the colour of the diseased Striga changed to dark brown or black (Fig. 4b). Browning of the infected seedlings started primarily at the place of connection (haustorium) to the sorghum root, then extended to the growing points, until they wilted and died as a result of the fungal infection.

Throughout the course of the experiment, application of Foxy 2 caused a strong and highly significant increase in the number of diseased Striga seedlings on tolerant and susceptible sorghum varieties compared to the controls of both varieties (p < 0.05) (Table 1). The results of the factorial analysis confirmed the efficacy of Foxy 2 by showing highly significant diseases on both varieties (F = 104.27, df = 1, 7, p < 0.001). The percentage of diseased killed Striga shoots showed a rapid increase during the course of the experiment, whereas in control treatments the rate of disease attached Striga remained significantly lower (Table 1). After 15 days, there was a significant difference in percentage of diseased Striga between treated and control treatments in both the tolerant and the susceptible sorghum varieties. At this stage, all Striga seedlings were totally healthy i.e., no disease symptoms were recorded, in the control treatments of both varieties, while in Foxy 2-treated tolerant and susceptible varieties 8.5% and 6% diseased seedlings, respectively, were counted.

Twenty-one days after sowing, the combination of Foxy 2 with the tolerant and susceptible varieties caused 56% and 41% disease on Striga seedlings, respectively (Table 1), whereas, their respective controls have only 14%, and 2%. The results showed a similar tendency after 26 days of sowing i.e. that Foxy 2 treated sorghum of the tolerant variety had 95% diseased Striga compared to 86% recorded in the case with the susceptible variety. On the same evaluation date, however, only 27% and 14% seedlings were dead in the control treatments of the tolerant and susceptible varieties, respectively (Table 1).

After 26 days of germination, the main effects of type of sorghum variety (tolerant and susceptible) on either sorghum root
length or total number of attached *Striga* seedlings were not significant (*p > 0.05*) (*F* = 2.62, df = 1, 7, *p* = 0.132, for root length and *F* = 2.70, df = 1, 7, *p* = 0.126, for total number of *Striga*). Similarly, the interactions between fungal mycoherbicide (factor I) and sorghum variety (factor II) were not significant (*p > 0.05*) for the total number of attached *Striga* (*F* = 0.083, df = 1, 15, *p* = 0.777), for the total number of diseased *Striga* (*F* = 0.127, df = 1, 15, *p* = 0.728) and for the sorghum root length (*F* = 0.063, df = 1, 15, *p* = 0.805). However, both control and Foxy 2-treated susceptible sorghum variety appeared to have higher numbers of attached *Striga* seedlings than the tolerant one (Table 1). Although, there was no statistical significant difference (*p > 0.05*) between Foxy 2-treated tolerant and susceptible varieties in causing disease symptoms on the attached *Striga* seedlings (Table 1).

### 4. Discussion

Proper delivery and timely establishment of the potential biocontrol agent *F. oxysporum* f. sp. *strigae* “Foxy 2” in the appropriate infection zone of *Striga* is necessary for ensuring a high biocontrol efficacy and facilitating field application and integration. For environmental reasons (minimization of exposure) as well as for the desire to extend the effect of the treatment beyond the germination, there is an increasing interest in biological control of seed- and soil-borne diseases by treating seeds with antagonistic microorganisms (Jenson and Wolhechel, 1992), for which microbial agents must be able to grow from the seed and colonize the developing root system in order to be effective. Delivering the potential *Striga*-mycoherbicides by seed treatment (Elzein et al., 2006), which is applicable to all varieties of cereal crops attacked by *Striga*, and compatible with other *Striga*-control methods, could accelerate the adoption and implementation of this biocontrol option by farmers.

#### 4.1. Colonization of Foxy 2 in sorghum roots

By coating the sorghum seeds with Foxy 2, the fungus has the opportunity of being the first colonizer of the host roots and then subsequently attacking *Striga* already during its attachment phase. The seed cover of gum arabic with its high sugar content (Grieve, 2007) appears to have provided a suitable nutrient source for Foxy 2 to start the colonization of the root system. Our cytological investigations showed that Foxy 2 was a slow but constant colonizer of roots. While only few hyphae were detectable on roots after one week, there were thick mycelia on the upper parts getting thinner versus the root tips after two to three weeks. Probably, the growth of the roots was faster than the growth of the mycelia. A similar observation was made on roots of tomato (*Lycopersicon lycopersicum*) by Olivain et al. (2006). By investigating the colonization of a pathogenic and a non-pathogenic strain of *F. oxysporum*, they found that both strains were able to colonize the entire root surface, with the exception of the apical zone. This is in contrast to investigations of Turlier et al. (1994) who found that the pathogenic strain *F. oxysporum* f. sp. *lini* Snyder & Hansen was entering the apical zone where the endodermal layer was not yet developed and the xylem tissue became infected when it was an undifferentiated group of cells just formed from subapical initials.

Foxy 2 was not only colonizing the root surface, it was also colonizing the cortical parenchyma of the host roots. In the root cortex hyphae were growing in the intercellular space, but also penetrating parenchyma cells and digesting cell walls and cytoplasm step by step. But Foxy 2 hyphae were not able to pass the endodermal layer to enter the tissues of the central cylinder. Transmission electron microscopy revealed that the central cylinder of the roots showed intact vessels and xylem parenchyma with functional cytoplasm. Hyphae did not penetrate the thickened walls of the endodermal cells and were never found in the central cylinder of the root even after four weeks. This is in contrast to pathogenic *Fusarium* strains which cause typical disease symptoms of tracheomycosis, e.g. wilting, chlorosis, and necrosis, by invading the central cylinder, infecting xylem parenchyma, and colonizing vessels. For example, the pathogen *F. oxysporum* f. sp. *radicis-lycopersici* Jarvis & Shoemaker was penetrating the endodermis of tomato roots between 96 and 120 h after inoculation, and 24 h later the central cylinder was colonized (Charest et al., 1984).

Foxy 2 acts like other non-pathogenic *Fusarium* strains by colonizing the root surface and the cortex of roots (Olivain et al., 2003; Paparú et al., 2006). Already Olivain and Alabouvette (1997) showed that *Fusarium* strains were able to colonize the cortex of roots to some extend. There are variations in the ability of non-pathogenic *Fusarium* strains to colonize host tissue. While *F. oxysporum* strain Fo47 colonized the root surface and infected only few cortical cells in tomato (Olivain and Alabouvette, 1997), in flax (*Linum usitatissimum* L.) (Olivain et al., 2003) the same strain was able to colonize cortex and central cylinder without disease symptoms in young seedlings of *Eucalyptus viminalis* Labill. (Salerno et al., 2000). There are reports that non-pathogenic fungi cause intense defence reactions such as wall appositions, a general wall thickening, accumulation of osmophilic material, collapsed cells, or intercellular plugging (Olivain et al., 2003). In the present study, there were no defence reactions in host cells visible.

Although, Foxy 2 was digesting the cortex of roots, there were no negative effects to the growth of the sorghum plants. Instead the Foxy 2-colonized plants developed a stronger root system than the controls. It might be that the reaction of the plant to the destruction of the root cortex is an enhanced growth of the root system and, especially the development of side-roots.

The cytological investigations showed that Foxy 2 is present in sorghum roots over a long period of time. The colonization of Foxy 2 started with seed germination, and hyphae were still in the root cortex after four weeks, when roots were already in the state of senescence. Foxy 2 hyphae metabolised step by step the cortical parenchyma cells. They were active and ready to attack *Striga* seedlings over this long period of time being active in and around roots,
the site where Striga seedlings attach. While it did not cause disease symptoms in the crop sorghum it was highly pathogenic to its specific target weed Striga by completely destroying it. Therefore, Foxy 2 applied as seed treatment is a promising biocontrol agent.

4.2. Non-colonization of Foxy 2 in sorghum shoots

Foxy 2 did not colonize the shoots of sorghum as its hyphae were not detected in the xylem vessels in sorghum shoots even after 11 weeks of sowing. This indicates that hyphae did not enter the vascular tissue of the root to grow through the vessel into the shoot of sorghum as pathogenic Fusarium species would do, and thus further proving evidence of the non-pathogenic behaviour of Foxy 2 to sorghum even at a later stage of growth. Furthermore, our results showed that coating with Foxy 2 did not cause any direct or indirect negative effects on germination and growth of the non-target plant sorghum. Likewise, Elzein and Kroschel (2006a) and Marley et al. (2006) reported immunity of all sorghum cultivars tested in host range studies; none has developed any symptoms of disease infection (e.g. pathogenic F. oxysporum strains cause wilting, dieback, necrosis and chlorosis), and no direct or indirect negative effects on their vegetative growth parameters including number of leaves, plant height, photosynthetic rate, and root and shoot biomass, were recorded after heavily inoculation with F. oxysporum f. sp. strigae (Foxy 2 and PSM197) inoculum.

4.3. Efficacy of Foxy 2-colonized roots in controlling Striga

The presence of the fungus along sorghum roots, on the surface and in the cortex, enables it to act effectively against Striga parasitism during the parasite haustorial initiation and establishment of vascular connections with host roots. The application of Foxy 2 by seed treatment on sorghum seeds showed high efficiency against S. hermonthica even after one year of storage as it caused disease in >86% of Striga seedlings attacking sorghum roots, in both Striga susceptible and tolerant varieties (Fig. 4b). This high efficacy of Foxy 2 is related to its high pathogenicity to Striga and its ability to colonize and spread along roots of sorghum seedlings. Because the life cycle of Striga is intimately linked to that of its host, timing and extent of colonization of the host roots by the fungus is of utmost importance for ensuring a significant parasitoid control. Preconditioning, germination, haustorial initiation, development and attachment, and establishment of vascular connections of the parasite take place in the vicinity of host roots and require 18–29 days depending on the species and environmental conditions (Rich and Ejeta, 2007). On the other hand, the age of sorghum roots is also a crucial factor in Striga parasitism. Maximum Striga germination and attachment was reported to take place when sorghum was 3–4 weeks old (Dawoud, 1995). The microscopic investigations confirmed the establishment and long presence of Foxy 2 along the root surface and in the cortex of sorghum during second to fourth week after germination. Thus the peak of number of Striga that get attached to host roots coincides with a strong host root colonization by Foxy 2, enabling the fungus to infect nearly all attached Striga seedlings. The fact that Foxy 2 is virulent against all developmental stages of S. hermonthica including seeds (Kroschel et al., 1996) makes the spreading of fungal mycelia at some distance from the root and in the rhizosphere necessary. An indication of the spread outside the root surface is that seeds and germ tubes of germinated Striga became infected even the development of attachment to the host root (Elzein et al., 2006). Thus, these advantages make the delivery of Foxy 2 by seed treatments appropriate and efficient for causing high and sustainable depletion of the weed seed bank taking into account the high fecundity and longevity of parasite seeds in soils (up to 15 years) (Parker and Riches, 1993).

4.4. Integrating Foxy 2 and Striga-tolerant sorghum

Striga resistant (low infestation with Striga) and tolerant (high yield in spite of Striga infestation) sorghum and maize varieties play an important role in integrated Striga management because their application is easy and supposed to be the most cost effective and environment-friendly method in Striga control for farmers (Gupta and Lagoke, 2000; Ejeta and Butler, 1993; Ejeta, 2007). Throughout the course of the experiment, application of Foxy 2 as film-coats on Striga-tolerant “Wad Ahmed” and susceptible “Cowbaula” sorghum varieties caused highly significant increases in the number of diseased Striga seedlings. Foxy 2 caused an increased disease incidence in Striga seedlings over time especially, indicating that Foxy 2 and the tolerant variety can make additive effects against early developmental stages of Striga. However, the difference between a Striga-tolerant and the susceptible sorghum variety were small and non-significant and need to be tested under field conditions where other parameters such as number of emerged Striga plants and yield components are also considered. The sorghum variety “Wad Ahmed” has been identified as tolerant/resistant to S. hermonthica based on a low number of emerged Striga plants and high grain yields under field conditions of Sudan (Prof. A.G. Babiker, Agricultural Research Corporation, Sudan, pers. com.). The higher percentage of diseased Striga seedlings in combination with the tolerant variety in the control treatment could be attributed to its ability to tolerate further Striga development by possessing some degree of resistance such as post-attachment hypersensitive reactions with Striga or through antibiotic, i.e. reduction of Striga development through unfavourable phytotormone supply by the host (Haussmann et al., 2000). This incompatibility may be weakened the Striga parasite, which become more susceptible to the fungal infection. The evidence that the sorghum variety “Wad Ahmed” possesses a resistant mechanism was given by Hiraoka and Sugimoto (2008). These and other authors (Mohamed et al., 2003) further described that the roots of the tolerant Wad Ahmed frequently became reddish in response to Striga attack, which was also observed in our studies. Furthermore, the premature death of Striga under natural conditions is a known phenomenon, which may be in part due to competition for nutrients, but its exact cause still remains unknown. The recorded dead Striga in the control treatments could be in part due to the same causes.

In recent reports, the impact of Striga resistant sorghum cultivars showed synergies when used in combination with other agronomic interventions (Mb wagona et al., 2007) or with a mycoherbical approach (biocountrol) (Schaub et al., 2006; Elzein et al., 2007) in an integrated Striga management program. Therefore, scaling up of Foxy 2-seed treatment technology to support and enhance the existing Striga control measures could facilitate large-scale application of Striga-mycoherbicides in Africa.

In conclusion, the temporal-pattern and extent of colonization and association of Striga-mycoherbicide Foxy 2 with sorghum roots supports the efficiency and appropriateness of seed treatment delivery system for ensuring a high biocontrol efficacy. The cyto logical proof of sorghum non-pathogenicity of the fungus may encourage the acceptance and field application of Foxy 2 by authorities and farmers. The compatibility, efficacy of Striga-mycoherbicide Foxy 2 with Striga resistant/tolerant sorghum using seed treatment indicates that effective, adoptable and environmentally friendly integrated control strategies for Striga can be successfully realized.

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