An explicit AFLP-based marker for monitoring *Fusarium oxysporum* f.sp. *strigae* in tropical soils

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Abstract

Our objective was to develop an explicit AFLP-marker to quantify the abundance of *Fusarium oxysporum* f.sp. *strigae* (Fos), an effective soil-borne biocontrol agent (BCA) against the parasitic weed *Striga hermonthica*, in tropical soils. The specificity of the AFLP-marker to Fos was confirmed on basis of comparison of *Fusarium* isolates of differing relatedness to Fos. These consisted of 40 Fos, 17 *Fusarium* spp. and 68 *F. oxysporum* isolates retrieved from tropical and temperate ecosystems. The robustness of the AFLP-marker for monitoring Fos was validated in a controlled incubation experiment. In this experiment, we inoculated the known Fos-BCA “Foxy-2” as model organism via seed coating (1.15×10^5 colony forming units per seed) of a tropical maize (*Zea mays* L.) variety to a tropical clayey (Humic Nitisol) and a sandy (Ferric Alisol) soil, both with no indigenous Fos infestation. The proliferation of “Foxy-2” was followed at 14, 28 and 42 days after start of experiment by Fos-specific quantitative PCR using the AFLP-marker. Moreover, the experimental set-up considered two additional factors: (1) presence of *S. hermonthica*, and (2) application of *Tithonia diversifolia* residues as nitrogen-rich resource for supporting “Foxy-2” proliferation. The explicit AFLP-marker was appropriate to reveal that soil type and organic resource availability exhibited distinct effects on abundance of inoculated “Foxy-2”. Negative PCR signals, which confirmed the Fos-specificity of the developed AFLP-marker, were retrieved from control soils not inoculated with “Foxy-2”. Hence, we verified the applicability of the explicit AFLP-marker for Fos in two soils, but recommend additional broad scale field studies to approve its suitability as Fos monitoring tool for differently managed soils in contrasting tropical agro-ecological zones.

Keywords: *Fusarium oxysporum* f.sp. *strigae*; *Striga hermonthica*; Biological control agent; AFLP; molecular marker; quantitative PCR; monitoring.

1. Introduction

The parasitic weed *Striga hermonthica* (Del.) Benth. is a major constraint to cereal production in Sub-Saharan Africa affecting the livelihood of about 100 million people (Ejeta, 2007). *S. hermonthica* lives parasitically on cereal crops such as millet (*Pennisetum americanum* L. Leeke), sorghum (*Sorghum bicolor* L.), maize (*Zea mays* L.), and rice (*Oryza sativa* L.) (Elzein and Kroschel, 2004; Marley et al., 2004). It causes annual crop damage ranging from 30 to 90% deemed equivalent to approximately 9 billion US dollars financial loss (Parker and Riches, 1993; van Mourik, 2007; Watson et al., 2007). Control of *S. hermonthica* remains challenging due to its extremely high seed production per plant with seed survival rates in soils of more than 10 years (Parker and Riches, 1993; van Mourik, 2007).

Current *S. hermonthica* control strategies include hand pulling, catch crops (e.g., Sudan grass (*Sorghum sudanense* L.)) and trap crops (e.g., soybean (*Glycine max* L.), lucerne (*Medicago sativa* L.), cotton (*Gossypium* spp.)), as well as the use of tolerant crop varieties. But these approaches were shown to be ineffective when applied individually. Hence, integrated approaches are postulated as control strategies against *S. hermonthica* (Atera et al., 2012; Hearne, 2009; Menkir and Kling, 2007).

Combination of biological control agents (BCAs) such as *Fusarium oxysporum* f.sp. *strigae* (Fos) strains along with tolerant crop varieties has shown respectable control success in field experiments conducted in Burkina Faso, Benin and Nigeria (Schaub et al., 2006; Venne et al., 2009). In this respect, the Fos strain “Foxy-2” was approved to be superior in suppressing all development 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 saprophytically the roots of cereals, where the biocontrol activity of “Foxy-2” was initialized after *S. hermonthica* attacked the root system. Hence, integration of Fos with tolerant crop varieties is, in comparison to the use of agrochemicals, an environmentally friendly and durable strategy to combat *S. hermonthica*.

Schaub et al. (2006) and Venne et al. (2009) showed, however, that the success of “Foxy-2” in controlling *S. hermonthica* under field conditions is variable, presumably as a consequence of differing environmental conditions across agro-ecological zones with different soil types, as well as rainfall and temperature patterns. Thus, if a prospective broad scale application of BCA Fos in agricultural fields infested by *S. hermonthica* is anticipated, its proliferation and survival potential in differently managed soil ecosystems requires a thorough evaluation. However, a specific monitoring tool is still lacking allowing the robust quantification of Fos abundance in target environments and also quality assessment during inoculum production. Conventional techniques for monitoring fungi in soils include the estimation of colony forming units (cfu) on selective media and immunological assays (e.g., ELISA) (Lievens and Thomma, 2005). These methods are generally time-consuming, require taxonomical expertise, and often lack in specificity for the organism of interest (Ward et al., 2004). Molecular, DNA-based techniques compensate many of known limitations of such conventional analyses. Recently, application of quantitative polymerase chain reaction (qPCR) emerged as a suitable method for specific monitoring of microorganisms in soils (Edel-Hermann et al., 2011; Jiménez-Fernández et al., 2010; Providenti et al., 2009). A number of qPCR assays were developed previously for *Fusarium* spp. (Bluhm et al., 2004; Pasquali et al., 2006; Waalwijk et al., 2004). Edel-Hermann et al. (2011) developed a strain-specific qPCR assay based on a sequence-characterized amplified region marker (SCAR) for quantification of BCA “Fo47”, a soil-borne *F. oxysporum* strain.

Amplified fragment length polymorphisms (AFLP; Vos et al., 1995) was developed as one of the most recognized DNA fingerprinting techniques to analyze fungal populations including *Fusarium* spp. in soils (e.g., Abd-Elsalam et al., 2004; Kiprop et al., 2002; Leslie et al., 2005; Silva et al., 2013). The reproducibility and resolution of AFLPs are superior to those of other markers (e.g., random amplified polymorphic DNAs, restriction fragment length

polymorphisms, microsatellites) (Mueller and Wolfenbarger, 1999). AFLP-markers for Fos as necessary prerequisite for monitoring its proliferation in soils are yet to be developed.

Our objective was thus to develop an explicit AFLP-marker for monitoring the abundance of Fos in soils via qPCR. The developed AFLP derived marker was tested for its specificity against 40 Fos, 17 *Fusarium* spp. and 68 *F. oxysporum* isolates and validated in a controlled incubation experiment. This experiment included two contrasting tropical soils (sandy Ferric Alisol versus clayey Humic Nitisol), with and without *S. hermonthica* infestation, to which “Foxy-2” was inoculated and its proliferation monitored during 42 days. Soils were not sterilized to validate the specificity of the monitoring tool against the indigenous soil fungal population and to monitor the “Foxy-2” proliferation under natural conditions. Additionally, an organic fertilization treatment with *Tithonia diversifolia* residues, a widely used green manure in Sub-Saharan Africa (Gachengo et al., 1998; Jama et al., 2000), was included to investigate its stimulating effect on the saprophytic survival of “Foxy-2” in studied soils.

2. Material and methods

2.1 AFLP marker development

2.1.1 Fungal strains, growth conditions and DNA extraction

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”. Details on the origin of *Fusarium* spp. and *F. oxysporum* isolates involved in this study are given in Table 1. Fos isolates CAV 6021 – CAV 6236 (Table 1) were recently collected from diseased *S. hermonthica* plants in Kenya and Nigeria, and pathogenicity tests were conducted to confirm their pathogenicity to *S. hermonthica* plants (H. Wainwright and A. Elzein, unpublished data). Fos isolates were further characterized by vegetative compatibility group (VCG) testing and phylogenetic analysis of Tef-1 α and

MtSSU DNA sequences (M. de Klerk, unpublished data). Strains were propagated in potato dextrose broth at 28°C for 3 days, followed by DNA extraction (UltraClean Microbial DNA Isolation Kit, MO BIO Laboratories Inc., Carlsbad, CA). Concentration and quality of isolated DNA were determined on a Nanodrop ND-1000 (Nanodrop Technologies, Wilmington, DE, USA).

2.1.2 Amplified fragment length polymorphism (AFLP) procedure

All primers (Biomers GmbH, Ulm, Germany) used are listed in Table 2. A selection of 9 *Fos* and 6 *F. oxysporum* isolates out of 125 isolates (Table 1) were included in AFLP fingerprinting to reduce labor and costs.

AFLP fragments were generated as described by Vos et al. (1995) with minor modifications. Digestion was performed at 37°C for 3 h using 300 ng genomic DNA extracted from each fungal isolate, 1x NEBuffer 4 (New England Biolabs Inc. (NEB), Ipswich, MA, USA), 1 U *Mse*I (NEB), 1 U *Eco*RI (NEB), 0.1 mg ml⁻¹ bovine serum albumin (NEB), and then adding water to a total volume of 50 µl. Preparation of the *Eco*RI adapter was done with 100 pmol *Eco*RI-ad forward, 100 pmol *Eco*RI-ad reverse and 1x Buffer C (Promega GmbH, Mannheim, Germany). Preparation of the *Mse*I adapter was conducted with 950 pmol *Mse*I-ad forward, 950 pmol *Mse*I-ad reverse and 1x Buffer C. Both adapter reactions were incubated at 96°C for 5 min, and cooled down slowly to room temperature. T4 DNA Ligase buffer (1x, NEB), 1 U T4 DNA Ligase (NEB), 1.5 µl *Eco*RI adapter and 1.5 µl *Mse*I adapter were mixed and filled up to 10 µl with water. Ten µl ligation mix were added to each restriction digestion mix and incubated at 16°C for 12 h. From each ligation, 2 µl were used as template for pre-selective PCR (1x PCR buffer, 1.5 mM MgCl₂, 0.25 mM dNTPs, 0.25 µM *Eco*RI pre-selective primer (*Eco*RI-0), 0.25 µM *Mse*I pre-selective primer (*Mse*I-0), and 1 U of *Taq* polymerase (Bioline GmbH, Luckenwalde, Germany) in a total volume of 20 µl). Pre-selective PCR started with 72°C for 2 min and 94°C for 2 min, which was followed by 26 cycles of 94°C for 1 min, 56°C

for 1 min, and 72°C for 1 min. A final extension at 72°C for 5 min completed the reaction. Pre-selective amplicons were diluted 5-fold for subsequent selective PCR. Selective primers were *EcoRI*-ACT and *MseI*-AA, and the PCR mix was similar to that used for pre-selective PCRs with 2 µl diluted template. Selective PCR included an initial denaturation of 94°C for 2 min, which was followed by 15 cycles of 94°C for 30 s, 65°C for 30 s (with a decrease of 0.7°C in each successive cycle), and 72°C for 1 min; then 25 cycles each of 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min; with a final extension at 72°C for 2 min. Amplicons were visualized with GelRed™ (Biotrend Chemikalien GmbH, Cologne, Germany) staining following electrophoresis (90 V, 3 h) in an agarose-synergel (0.7% agarose and 0.5% Synergel™ (Carl Roth GmbH, Karlsruhe, Germany)).

Target bands in the AFLP fingerprint gel (highlighted in Fig. 2) were excised and purified (QIAquick Gel Extraction Kit, Qiagen GmbH, Hilden, Germany). Since DNA quantity after purification from agarose gel was very low, purified DNA was used as template for an additional PCR (2 µl template DNA, 1x PCR buffer, 2 mM MgCl₂, 0.25 mM dNTPs, 0.25 µM *EcoRI*-ACT, 0.25 µM *MseI*-AA, and 1 U of proofreading high fidelity Velocity polymerase (Bioline GmbH)) with the same PCR conditions as used for selective PCR in AFLP (see above). Amplicons were checked for correct band size on a 1.5% agarose gel and purified (Invisorb® DNA CleanUP Kit, Stratec Molecular GmbH, Berlin, Germany). DNA concentration of purified amplicons was determined as described above and was adjusted to the recommended DNA concentration for sequencing. Diluted amplicons were sequenced with the *MseI*-AA primer at LGC Genomics GmbH (Berlin, Germany).

2.1.3 Marker design

Obtained DNA sequences were analyzed using BLAST (Altschul et al., 1990) of the National Centre for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>) and the *Fusarium* database (*Fusarium* Comparative Sequencing Project, Broad Institute of Harvard and MIT) to

determine if any sequence homologues exist in databases. Primers targeting internal sequences of AFLPs were designed using Primer-BLAST (<http://www.ncbi.nlm.nih.gov/tools/primer-blast>) in consideration of relatively high annealing temperatures ($\geq 60^{\circ}\text{C}$) and amplicon sizes smaller than 200 bp to reduce generation of artifacts and to achieve proper reaction efficiencies during qPCR. The designed primer pair (Kb1::Kb2, Table 2) generated an amplicon of 165 bp with the following sequence: 5'-GGACGAACTGACAGCCCTACGAGAATGGCTTGATGAGAACCTACGGAAAGGGTTTATCAGGCCAAGCTCCTCCCCCGTGGCATCCCCTGTGCTGTTCGTAAAGAAGCCA GGAGGAGGACTACGATTCTGCGTAGACTACAGAGCTCTGAACAATATTACGGTTA C-3'.

2.1.4 PCR protocol for the developed marker

The primer pair was first tested for its specificity on all isolates (i.e., 40 *Fos*, 17 *Fusarium* spp. and 68 *F. oxysporum* isolates (Table 1)). Five ng of the *Fusarium* isolates DNA was used as template for PCR (1x PCR buffer, 1.5 mM MgCl_2 , 0.25 mM dNTPs, 0.25 μM of each primer and 1 U of *Taq* polymerase (Bioline GmbH) in a total volume of 20 μl). PCR started with 94°C for 2 min, followed by 35 cycles of 94°C for 1 min, 65°C for 30 s, and 72°C for 30 s. Reaction was completed with a final extension at 72°C for 2 min. Amplicons were visualized with GelRedTM (Biotrend Chemikalien GmbH) staining following electrophoresis (120 V, 45 min) in a 1.5% agarose gel. In a second step, the developed primer set was validated in DNA extracts obtained from soils of the incubation experiment (section 2.2) with the PCR protocol described above using 10 ng soil DNA as template.

2.2 Rhizobox experiment

A rhizobox experiment was conducted to validate the developed *Fos* marker in two contrasting tropical soils. Details on the set-up of the rhizobox can be retrieved from Musyoki

et al. (2015). The rhizobox experiment was arranged as a completely randomized design with 6 treatments with 3 replicates each: 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), and iv) coated maize seeds with “Foxy-2” and *S. hermonthica* (F+S), v) coated maize seeds with “Foxy-2” and *T. diversifolia* (F+T), as well as vi) coated maize seeds with “Foxy-2”, *S. hermonthica* and *T. diversifolia* (F+S+T).

Rhizosphere soil samples were taken 14, 28 and 42 days after planting (DAP). 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 destruct the rooting system. Rhizosphere soil was gently scrapped off with sterile forceps and transferred into sterile sampling bags. Chemical soil analyses were done using bulk soil samples at 42 DAP. Rhizosphere soil samples for molecular analysis were freeze-dried and stored at -20°C, while bulk soils for chemical analyses were kept at -20°C without freeze drying.

2.3 Molecular and chemical analysis of soil samples

2.3.1 DNA extraction from rhizosphere samples

Total genomic DNA from rhizosphere soil samples was extracted using the Fast DNA® Spin Kit for Soil (MP Biomedicals, Solon, OH, USA) following the manufacturer’s instructions with slight modifications. Briefly, 0.4 g freeze-dried soil was bead-beated for 30 s with a beating power of 5.5 m s⁻¹ using a FastPrep®-24 Instrument (MP Biomedicals). Extracted DNA was quantified as described above. A soil spiking experiment was included to account for soil type depending DNA extraction efficiencies influencing “Foxy-2” gene copy recovery: 400 mg of freeze dried soil samples obtained from control sets of the rhizobox experiment were transferred into the beat beating tubes of the DNA extraction kit (MP Biomedicals). Soil samples in tubes were spiked with cloned “Foxy-2” amplicons of known

concentration (10^3 “Foxy-2” gene copies). Recovery of “Foxy-2” amplicons after DNA extraction was determined using the qPCR protocol described in section 2.3.2.

2.3.2 Quantitative PCR protocol for the developed AFLP marker

Each reaction (20 μ l) contained 10 ng rhizosphere soil DNA template, 10 μ l of Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 0.2 μ M of each primer Kb1 and Kb2, as well as 0.2 μ l T4 gene 32 protein (500 ng μ l⁻¹, MP Biomedicals). A cloned amplicon was used as standard in 10-fold serial dilutions of known DNA concentration. PCR runs were performed on a StepOnePlus™ Real-Time PCR System (Applied Biosystems). Reactions started with initial denaturation at 95°C for 10 min, followed by 45 cycles of denaturation at 94°C for 30 s, annealing at 65°C for 30 s, and polymerization at 72°C for 1 min as well as one additional step at 77°C for 30 s for signal detection. Occasionally, small peaks occurred in the melting curve between 72 and 76°C due to primer dimers not detected by electrophoresis in a 1.5% agarose gel (data not shown). To avoid measurement of fluorescence signal emitted by these primer dimers, fluorescence of target amplicon (melting temperature (T_m) = 81.8°C) was detected at 77°C. Each DNA sample was processed in triplicate reactions, while standards were run in duplicates. Melting curve analysis of amplicons was conducted to confirm that fluorescence signals originated from specific amplicons and not from primer dimers or other artifacts. Quantification of gene copies was calculated by comparing values of threshold cycles (C_t) to values of crossing points of the linear regression line of the standard curve using StepOne™ software version 2.2 (Applied Biosystems).

2.3.3 Measurement of soil chemical parameters

Total carbon (TC), total nitrogen (N_t), extractable organic C (EOC), extractable N (EON) and pH were recorded using bulk soils from each rhizobox shortly taken at the last sampling date

(DAP 42). The pH analysis was conducted in a soil water ratio of 1:2.5 (inoLab® Labor-pH-Meter, WTW GmbH, Weilheim, Germany). TC and N_t were measured by total combustion (vario MAX CN analyzer, Elementar Analysensysteme GmbH, Hanau, Germany). For EOC measurement, 5 g of soil were extracted with 20 ml 0.5 M K_2SO_4 , shaken horizontally (250 rpm) for 30 min and filtered (Rotilabo-Rundfilter AP55.1 with a retention of 2-3 μm , Carl Roth GmbH). EOC concentration in each filtered extract was measured on an Analytik Jena Multi N/C 2100 analyzer (Analytik Jena AG, Jena, Germany). Ammonium (NH_4^+) and nitrate (NO_3^-) were extracted with 1 M KCL (soil-to-extractant ratio (w/v) of 1:4), shaken on a horizontal shaker for 30 min at 250 rpm and filtered (Rotilabo-Rundfilter AP55.1). Concentrations of NH_4^+ and NO_3^- were measured on an auto-analyzer (Bran & Luebbe, Norderstedt, Germany). EON was calculated as follows: $EON = N_t - (NH_4^+ + NO_3^-)$.

2.4 Statistical analysis

Each rhizobox was sampled at 3 sequential dates (DAP 14, 28 and 42). Hence, a repeated measures analysis with an autoregressive covariance structure using the “nlme” package (Pinheiro et al., 2014) combined with post hoc Tukey-B tests using the “lsmeans” package (Lenth, 2013) in the statistic software R (R Core Team, 2013) was performed to determine effects of “*S. hermonthica*”, “*T. diversifolia*”, “Soil type” and “Sampling date” on abundance of “Foxy-2”. For this, obtained “Foxy-2” gene copy numbers were square-root transformed to meet the assumptions of parametric statistical tests. Pearson’s correlation coefficients were used to assess significant relations between “Foxy-2” abundance and soil chemical parameters.

3. Results

3.1 Development of the molecular monitoring tool for BCA Fos

3.1.1 AFLP fingerprint

In an initial test, several primer combinations with different selective primer extensions (Vos et al., 1995) were evaluated on their ability to separate the Fos group from other *Fusarium* spp. and *F. oxysporum* strains via AFLP. Selective primer extensions were suited for visual fingerprint comparison resulting in a 3 base-pair extension of the selective *Eco*RI-primer and a 2 base-pair extension of selective *Mse*I-primer. Gel electrophoresis with 0.7% agarose + 0.5% synergel combination provided an optimal separation of fragments with 100 to 1000 bp. The fingerprint, from which the explicit marker for Fos finally resulted, was based on 15 out of 125 pre-selected isolates (Fig. 1). AFLP fingerprinting with primers *Eco*RI-ACT/*Mse*I-AA separated clearly the Fos group from the other 6 *F. oxysporum* isolates tested. The highlighted bands were excised, purified, sequenced and used for AFLP marker development. The suitability of the *Eco*RI-ACT/*Mse*I-AA fingerprint for marker development was substantiated against other primer combinations (e.g., *Eco*RI-TTG/*Mse*I-CA) from which no Fos group separating bands were identified (Fig. 2).

3.1.2 Primer specificity testing

The AFLP derived primer pair Kb1::Kb2 (Table 2) was checked for its specificity to Fos via PCR including 40 Fos, 17 *Fusarium* spp. and 68 *F. oxysporum* isolates belonging to 15 different species and 20 forma speciales (f.sp.) (Table 1; Fig. 3). The only non-Fos isolate amplifying with the developed primers was *F. oxysporum* f.sp. *melonis* (CAV 343, Table 1) originating from Israel. Its amplicon showed the same fragment length (165 bp) and DNA sequence as the Fos amplicon. All other non-Fos *Fusarium* spp. and *F. oxysporum* isolates, including the f.sp. *melonis* of African (CAV 317, Table 1) and European (*melonis*, Table1) origin, were not detectable with the developed PCR protocol used for specificity testing.

Alignment of the 165 bp amplicon sequence in BLAST using specifically MEGABLAST for highly similar sequences resulted in no match. With using discontinuous MEGABLAST for comparing diverged sequences, the highest match (76% identity) was found for *Magnaporthe*

oryzae covering 70% of the amplicon sequence. Further alignment in the *Fusarium* database showed the highest match (84% identity) with “*F. oxysporum* II5: Supercontig 200: 2407-2554” with a sequence coverage of 90%. Alignment of primer sequences Kb1 and Kb2 in Primer-BLAST resulted in one match with *Cryptococcus neoformans* (accession number: NC006685.1) showing an identity score of 80% to both primers Kb1 and Kb2 and generating an amplicon of 4113 bp length. This could be clearly distinguished from the desired Fos amplicon of 165 bp. Additionally, due to the fact that *C. neoformans* is not soil borne, it is considered not to interfere with the Fos monitoring tool for envisaged soil application. Further alignment of primer sequences Kb1::Kb2 in the *Fusarium* database, which includes among others the full genome sequence of one f.sp. *melonis* isolate (NRRL 26406, Ma et al., 2014), resulted in no match. This substantiated the specificity of the developed marker Kb1::Kb2 to Fos in target African soils.

3.1.3 Quantitative PCR protocol

Accurate standard curves were generated in the range of 4.66×10^5 - 10^1 “Foxy-2” gene copy numbers (Fig. 4), thus the detection limit of the qPCR assay was approximately 10 gene copies of “Foxy-2” per reaction. Standard regression lines approved, according to Smith and Osborn (2009), high quality performance (i.e., reaction efficiencies $\geq 94\%$, coefficients of determination (R^2) ≥ 0.997). Specificity of amplifications was verified by melting curve analysis (Fig. 4). Control sets without “Foxy-2” inoculation showed no amplification in qPCR confirming the specificity of the AFLP marker to Fos in the two model soils from Embu (clayey Humic Nitisol) and Machanga (sandy Ferric Alisol). Moreover, the soil spiking experiment confirmed the soil type independent “Foxy-2” DNA recovery after DNA extraction since no significant difference in “Foxy-2” gene copy numbers between the clayey and sandy soils was detected ($P > 0.05$).

3.2 Validation of the monitoring tool

Figure 5 displays the proliferation of “Foxy-2” in the rhizosphere soils of the 4 “Foxy-2” amended treatments at each sampling date (i.e., DAP 14, 28 and 42). In the non-inoculated control sets, Fos was not detected and these samples were then excluded from statistical analysis. Clear interactions were found between treatments “Soil type” and “*T. diversifolia*” ($P < 0.001$) as well as “Soil type” and “Sampling date” ($P < 0.01$). No *S. hermonthica* root attachment and emergence was detected in the rhizobox experiment over all treatments within the period of 42 days. Accordingly, the “*S. hermonthica*” treatment (F+S) did not induce any effect on “Foxy-2” gene copy numbers ($P > 0.05$) compared to the “Foxy-2” treatment (F). Treatments with *T. diversifolia* residues (F+T, F+S+T) promoted “Foxy-2” abundance in both soils ($P < 0.001$). In general, *T. diversifolia* treated sandy soils revealed a higher “Foxy-2” abundance than their clayey counterparts ($P < 0.001$). From day 28 onwards, treatment *T. diversifolia* in the clayey soils showed, in comparison to the non-treated soils, an increase of “Foxy-2” gene copy numbers ($P < 0.001$). In the sandy soils, a similar effect was detected from day 14 onwards ($P < 0.001$).

3.2.1 Correlation between “Foxy-2” gene copy numbers and soil chemical data

Pearson’s correlations were assessed between “Foxy-2” gene copy numbers and soil chemical data (Table 3) at DAP 42. Positive correlations were calculated between “Foxy-2” gene copy numbers and pH (clayey soils: $r = 0.706$, $P < 0.01$; sandy soils: $r = 0.931$, $P < 0.0001$) and total carbon (TC) (clayey soils: $r = 0.751$, $P < 0.001$; sandy soils: $r = 0.726$, $P < 0.001$) in both soils. In the sandy soils, “Foxy-2” gene copy numbers showed a positive correlation with extractable organic carbon (EOC) ($r = 0.894$, $P < 0.001$), total nitrogen (N_t) ($r = 0.747$, $P < 0.001$) and ammonium (NH_4^+) ($r = 0.823$, $P < 0.05$). Nitrate (NO_3^-) showed a negative correlation with “Foxy-2” gene copy numbers in the clayey soils ($r = -0.870$, $P < 0.01$).

4. Discussion

4.1 Development of the molecular monitoring tool for Fos

In the present study, we developed a qPCR assay that allows DNA-based quantification of Fos in soils. For this purpose, a Fos-specific DNA fragment was identified using PCR-based AFLP fingerprinting. Specificity of the generated AFLP marker was validated against 40 Fos and 85 non-Fos isolates representing 15 different species and 20 formae speciales (f.sp.). This was, compared to other studies (e.g., Cipriani et al., 2009; Dubey et al., 2013), an extensive collection of isolates of varied relatedness and was hence an essential fundament for obligatory marker specificity testing. Additional alignment of the AFLP marker sequence in BLAST and in the *Fusarium* database substantiated the specificity of the AFLP marker to Fos. However, one *F. oxysporum* f.sp. *melonis* (Fom) isolate originating from Israel (i.e., CAV 343) gave a positive PCR signal when using the newly developed Fos marker. This co-amplifying isolate was characterized previously to be a member of race 1.2 (Risser, 1976) belonging to the vegetative compatibility group (VCG) 0138 with its geographical restriction to Israel (Katan et al., 1994). Due to this geographical divergence, no interference of the Fom isolate with the Fos marker is expected in African soils for which our marker was specifically developed. Additional Fom isolates originating from Africa (CAV 317, Table 1) and Europe (*melonis*, Table1) were tested and these did not show a PCR signal on basis of the developed Fos-marker. Furthermore, the *Fusarium* database includes the full DNA sequence of a Fom isolate which did also provide no match with the Fos-marker. This clearly strengthened the Fos-marker specificity for envisaged application in target African soils.

4.2 Validation of the monitoring tool in soils

The negative signals retrieved in qPCR from control sets in the rhizobox experiment confirmed the specificity of the developed Fos-marker to the inoculated Fos-strain “Foxy-2” in the two tropical soils from Embu (clayey Humic Nitisol) and Machanga (sandy Ferric

Alisol). We showed that “Foxy-2” proliferated in the maize rhizosphere. Further, it was obvious that distinct soil chemical characteristics and particularly the application of *T. diversifolia* residues stimulated “Foxy-2” abundance. The *S. hermonthica* treatment did not pose a significant effect on “Foxy-2” proliferation which was related to the non-existent *S. hermonthica* root infection. This finding contrasted our hypothesis that the presence of the host *S. hermonthica* would stimulate the proliferation of “Foxy-2”. Hence, we argued that the duration of the rhizobox experiment (42 days) was probably not sufficient to observe the expected *S. hermonthica* effect on “Foxy-2” abundance. Therefore, we recommend further experiments under an extended time period to investigate the presumed interaction between *S. hermonthica* and “Foxy-2”.

The general assumption that sandy soils compared to clayey soils offer favorable growth conditions to fungi (Dominguez et al., 2001; Stotzky and Martin, 1963) was not observed in this study since no distinction in “Foxy-2” abundance was detected between the two soils throughout the experiment. Nevertheless, addition of *T. diversifolia* residues posed a stronger effect on propagation of “Foxy-2” in the sandy soils. Application of *T. diversifolia* residues was accompanied with pronounced increases in ammonium (NH_4^+) levels in the sandy than clayey soils. Accordingly, a positive correlation was detected between NH_4^+ content and “Foxy-2” gene copy number in the sandy soils. Nishio and Furusaka (1970) as well as Ranjard and Richaume (2001) have shown higher NH_4^+ levels in sandy soils as a result of lower colonization of nitrifying microorganisms. Analysis of nitrifying prokaryotes abundance in the same rhizobox experiment confirmed the lower abundance of nitrifying prokaryotes in the sandy soils as opposed to the clayey soils (parallel study of Musyoki et al., 2015). This fact induced obviously an advantageous resource availability for “Foxy-2” utilizing the released NH_4^+ from *T. diversifolia* residue decomposition (Rasche et al., 2014). Hence, supplementation of *T. diversifolia* residues offered a favourable environment for

saprophytic proliferation of “Foxy-2” which was pronounced in resource poor sandy soils with low abundance of nitrifying prokaryotes.

5. Conclusions

Our results manifested that AFLP derived markers provide a robust strategy to selectively and sensitively detect and enumerate microorganisms such as Fos in African soils via qPCR. We confirmed the presence and accuracy of the predicted PCR fragment via melting curve analysis and gel electrophoresis. Both approaches approved the specific amplification of the desired fragment as generated by the AFLP derived marker. With respect to this sensitivity, we found clear indications that propagation of Fos (i.e., “Foxy-2”) was driven by distinct physico-chemical soil characteristics and organic fertilization (saprophytic survival).

To further approve the specificity and application robustness of the Fos explicit AFLP marker in contrasting environments, in which Fos may proliferate differently in the long-term including its success in controlling *S. hermonthica*, we recommend specific validations of the qPCR-based monitoring tool in a broad variety of contrasting tropical soils of Africa and cropping systems including site-specific organic matter management strategies (e.g., use of organic residues such as *T. diversifolia*) (Rasche and Cadisch, 2013).

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strains isolated from *Dianthus caryophyllus*. Dr. Juan Carlos Laso Bayas (University of Hohenheim, Institute of Crop Science and Bioinformatics, Stuttgart, Germany) assisted in statistical analysis.

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Figure captions

Figure 1. AFLP fingerprint obtained for a selection of *Fusarium oxysporum* f.sp. *strigae* (Fos) and *Fusarium oxysporum* isolates with the primer pair *Eco*RI-ACT and *Mse*I-AA. Lane M shows the 100 bp ladder (Promega GmbH, Mannheim, Germany). Bands which were excised from gel and used for marker development are given in the black box. Numbers above lanes represent the following *Fusarium* isolates (codes described in Table 1): 1 = AC3, 2 = FOG, 3 = *melonis*, 4 = *lycopers*, 5 = ACC01, 6 = 308, 7 = “Foxy-2”, 8 = PSM197, 9 = M6-1A, 10 = M12-4A, 11 = N2-5A, 12 = K7-5A, 13 = B7-5A, 14 = M5-1B, 15 = N5-1A.

Figure 2. AFLP fingerprint obtained for a selection of *Fusarium oxysporum* f.sp. *strigae* (Fos) and *Fusarium oxysporum* isolates with the primer pair *Eco*RI-TTG and *Mse*I-CA. Lane M shows the EasyLadder I (Bioline USA Inc., Taunton, USA). Numbers above lanes represent the following *Fusarium* isolates (codes described in Table 1): 1 = AC3, 2 = FOG, 3 = *melonis*, 4 = *lycopers*, 5 = ACC01, 6 = 308, 7 = “Foxy-2”, 8 = PSM197, 9 = M6-1A, 10 = M12-4A, 11 = N2-5A, 12 = K7-5A, 13 = B7-5A, 14 = M5-1B, 15 = N5-1A.

Figure 3. Agarose gel picture shows the specificity testing of the developed Fos marker Kb1:Kb2 in PCR including 52 representative *Fusarium* isolates selected from table 1. Lane M indicates the 100 bp ladder (Promega GmbH, Mannheim, Germany). Numbers 1-26 represent Fos isolates with 1 = “Foxy-2”. Numbers 27-52 represent non-Fos isolates with 52 = CAV 343. NC = negative control.

Figure 4. Amplification plot of qPCR standards obtained from the developed Fos marker Kb1::Kb2 ranging from 4.66×10^5 to 10^1 Fos gene copies. Melting curve of qPCR standards

shows a $T_m = 81.5^\circ\text{C}$. As a small peak occurred at $T_m = 71^\circ\text{C}$ to 76°C due to primer dimers, the fluorescence signal for quantification of Fos was recorded at 77°C .

Figure 5. Abundance of Fos at 14 (A), 28 (B) and 42 (C) days after inoculation into the clayey Humic Nitisol (Embu) and sandy Ferric Alisol (Machanga) with the four treatments: coated maize seeds with “Foxy-2” (F), coated maize seeds with “Foxy-2” and *S. hermonthica* (F+S), as well as coated maize seeds with “Foxy-2” and *T. diversifolia* (F+T) and coated maize seeds with “Foxy-2”, *S. hermonthica* and *T. diversifolia* (F+S+T). Bars represent back-transformed means with upper and lower confidence limit (95% confidence interval). Different letters indicate significant differences in Fos gene copy number at $P < 0.05$.

Fos isolates

Amplification Plot

Melting Curve

Table captions

Table 1. Origin of *Fusarium* isolates used in this study.

Table 2. List of primers including the developed Fos marker (Kb1::Kb2) used in this study.

Table 3. Soil chemical properties (mean $\pm$ standard deviation) obtained from the rhizobox experiment at DAP 42. Different letters within columns indicate significant differences ($P < 0.05$).

Table 1.

Code	Species	Origin	Isolated from	Reference
Foxy-2	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Ghana	<i>Striga hermonthica</i>	Elzein et al. (2004)
M6-1A	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Mali	<i>Striga hermonthica</i>	Ciotola et al. (1995), Venne (2008)
PSM 197	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Nigeria	<i>Striga hermonthica</i>	Marley et al. (2004)
N2-5A	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Niger	<i>Striga hermonthica</i>	Ciotola et al. (1995), Venne (2008)
M5-1B	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Mali	<i>Striga hermonthica</i>	Ciotola et al. (1995), Venne (2008)
N1-2A	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Niger	<i>Striga hermonthica</i>	Ciotola et al. (1995), Venne (2008)
M8-5A	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Mali	<i>Striga hermonthica</i>	Ciotola et al. (1995), Venne (2008)
K7-5A	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Kenya	<i>Striga hermonthica</i>	Ciotola et al. (1995), Venne (2008)
N5-1A	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Niger	<i>Striga hermonthica</i>	Ciotola et al. (1995), Venne (2008)
B7-5A	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Burkina Faso	<i>Striga hermonthica</i>	Ciotola et al. (1995), Venne (2008)
M12-4A	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Mali	<i>Striga hermonthica</i>	Ciotola et al. (1995), Venne (2008)
FK1	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Kenya	<i>Striga hermonthica</i>	Wainwright H. (2014, unpublished data)
FK2	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Kenya	<i>Striga hermonthica</i>	Wainwright H. (2014, unpublished data)
FK3	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Kenya	<i>Striga hermonthica</i>	Beed et al. (2013)
FK4	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Kenya	<i>Striga hermonthica</i>	Wainwright H. (2014, unpublished data)
FK5	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Kenya	<i>Striga hermonthica</i>	Wainwright H. (2014, unpublished data)
CAV 6021	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Kenya	<i>Striga hermonthica</i>	de Klerk M. (2014, unpublished data)
CAV 6023	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Kenya	<i>Striga hermonthica</i>	de Klerk M. (2014, unpublished data)
CAV 6039	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Kenya	<i>Striga hermonthica</i>	de Klerk M. (2014, unpublished data)
CAV 6040	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Kenya	<i>Striga hermonthica</i>	de Klerk M. (2014, unpublished data)
CAV 6044	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Kenya	<i>Striga hermonthica</i>	de Klerk M. (2014, unpublished data)
CAV 6045	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Kenya	<i>Striga hermonthica</i>	de Klerk M. (2014, unpublished data)
CAV 6067	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Kenya	<i>Striga hermonthica</i>	de Klerk M. (2014, unpublished data)
CAV 6077	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Kenya	<i>Striga hermonthica</i>	de Klerk M. (2014, unpublished data)
CAV 6211	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Kenya	<i>Striga hermonthica</i>	de Klerk M. (2014, unpublished data)
CAV 6090	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Nigeria	<i>Striga hermonthica</i>	Elzein A. (2014, unpublished data)
CAV 6091	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Nigeria	<i>Striga hermonthica</i>	Elzein A. (2014, unpublished data)

CAV 6092	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Nigeria	<i>Striga hermonthica</i>	Elzein A. (2014, unpublished data)
CAV 6116	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Nigeria	<i>Striga hermonthica</i>	Elzein A. (2014, unpublished data)
CAV 6135	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Nigeria	<i>Striga hermonthica</i>	Elzein A. (2014, unpublished data)
CAV 6143	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Nigeria	<i>Striga hermonthica</i>	Elzein A. (2014, unpublished data)
CAV 6166	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Nigeria	<i>Striga hermonthica</i>	Elzein A. (2014, unpublished data)
CAV 6167	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Nigeria	<i>Striga hermonthica</i>	Elzein A. (2014, unpublished data)
CAV 6233	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Nigeria	<i>Striga hermonthica</i>	Elzein A. (2014, unpublished data)
CAV 6236	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Nigeria	<i>Striga hermonthica</i>	Elzein A. (2014, unpublished data)
573	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Benin	<i>Striga hermonthica</i>	Venne (2008)
690	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Burkina Faso	<i>Striga hermonthica</i>	Venne (2008)
782	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Burkina Faso	<i>Striga hermonthica</i>	Venne (2008)
783	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Burkina Faso	<i>Striga hermonthica</i>	Venne (2008)
784	<i>F. oxysporum</i> f.sp. <i>strigae</i>	Burkina Faso	<i>Striga hermonthica</i>	Venne (2008)
773	<i>Fusarium</i> spp.	Burkina Faso	<i>Striga hermonthica</i>	Yonly et al. (2005)
774	<i>Fusarium</i> spp.	Burkina Faso	<i>Striga hermonthica</i>	Yonly et al. (2005)
775	<i>Fusarium</i> spp.	Burkina Faso	<i>Striga hermonthica</i>	Yonly et al. (2005)
777	<i>Fusarium</i> spp.	Burkina Faso	<i>Striga hermonthica</i>	Yonly et al. (2005)
CAV 6017	<i>F. incarnatum-equiseti</i> species complex	Kenya	<i>Striga hermonthica</i>	de Klerk M. (2014, unpublished data)
CAV 6028	<i>F. equiseti</i>	Kenya	<i>Striga hermonthica</i>	de Klerk M. (2014, unpublished data)
CAV 6032	<i>F. solani</i>	Kenya	<i>Striga hermonthica</i>	de Klerk M. (2014, unpublished data)
CAV 6093	<i>F. semitectum</i>	Nigeria	<i>Striga hermonthica</i>	de Klerk M. (2014, unpublished data)
CAV 6056	<i>F. incarnatum</i>	Kenya	<i>Striga hermonthica</i>	de Klerk M. (2014, unpublished data)
CAV 191	<i>F. oxysporum</i> f.sp. <i>cubense</i>	Indonesia	<i>Musa L.</i>	Groenewald et al. (2006)
CAV 789	<i>F. oxysporum</i> f.sp. <i>cubense</i>	Australia	<i>Musa L.</i>	Sutherland et al. (2013)
CAV 528	<i>F. oxysporum</i> non pathogen	South Africa	<i>Musa L.</i>	Belgrove et al. (2011)
CAV 529	<i>F. oxysporum</i> non pathogen	South Africa	<i>Musa L.</i>	Belgrove et al. (2011)
CAV 095	<i>F. oxysporum</i> f.sp. <i>cubense</i> STR4	South Africa	<i>Musa L.</i>	FABI, Pretoria University, South Africa
CAV 311	<i>F. oxysporum</i> f.sp. <i>cubense</i> TR4	Indonesia	<i>Musa L.</i>	FABI, Pretoria University, South Africa
CAV 183	<i>F. oxysporum</i> f.sp. <i>cubense</i> race 1	Australia	<i>Musa L.</i>	Groenewald et al. (2006)
CAV 556	<i>F. oxysporum</i> non pathogen	South Africa	<i>Musa L.</i>	Belgrove et al. (2011)

CAV 315	<i>F. oxysporum</i> f.sp. <i>lycopersici</i>	South Africa	<i>Solanum lycopersicum</i>	PPRI 5456
CAV 317	<i>F. oxysporum</i> f.sp. <i>melonis</i>	South Africa	<i>Cucurbitaceae</i>	PPRI 4923
CAV 324	<i>F. oxysporum</i> f.sp. <i>niveum</i>		<i>Citrullus lanatus</i>	Viljoen A. (Stellenbosch University, SA)
CAV 326	<i>F. oxysporum</i> f.sp. <i>conglutin</i>	USA	<i>Brassica</i>	CBS 186.53
CAV 327	<i>F. oxysporum</i> f.sp. <i>dianthi</i>	Netherlands	<i>Carnation</i>	CBS 491.97
CAV 328	<i>F. oxysporum</i> f.sp. <i>gladioli</i>	Netherlands	<i>Gladiolus</i>	CBS 137.97
CAV 329	<i>F. oxysporum</i> f.sp. <i>lini</i>	Canada	<i>Linum usitatissimum</i>	CBS 259.51
CAV 331	<i>F. oxysporum</i> f.sp. <i>tulipae</i>		<i>Tulipa</i>	CBS 195.65
CAV 333	<i>F. oxysporum</i> f.sp. <i>phaseoli</i>	USA	<i>Phaseolus vulgaris</i>	CBS 935.73
CAV 334	<i>F. oxysporum</i> f.sp. <i>pisi</i>	UK	<i>Pisum sativum</i>	CBS 127.73
CAV 336	<i>F. oxysporum</i> f.sp. <i>raphanni</i>	Germany	<i>Raphanus sativus</i>	CBS 488.76
CAV 338	<i>F. oxysporum</i> f.sp. <i>elaeidis</i>	Suriname	<i>Elaeis guineensis</i>	CBS 783.83
CAV 339	<i>F. oxysporum</i> f.sp. <i>nicotianae</i>		<i>Nicotiana tabacum</i>	CBS 179.32
CAV 340	<i>F. oxysporum</i> f.sp. <i>passiflorae</i>	Brazil	<i>Passiflora edulis</i>	CBS 744.79
CAV 341	<i>F. oxysporum</i> f.sp. <i>perniciosum</i>	Iran	<i>Mimosa</i>	CBS 794.70
CAV 345	<i>F. oxysporum</i> f.sp. <i>chrysanthemi</i>	USA	<i>Chrysanthemum</i>	CBS 129.81
MRC 2301	<i>F. proliferatum</i>	USA	<i>Zea mays</i>	Marasas et al. (1986)
MRC 0115 a	<i>F. subglutinans</i>	South Africa	<i>Zea mays</i>	Marasas et al. (1986)
NRRL 31084	<i>F. graminearum</i>	USA	<i>Zea mays</i>	Cuomo et al. (2007)
MRC 8267	<i>F. verticillioides</i>	South Africa	<i>Zea mays</i>	Fandohan et al. (2005)
CAV 337	<i>F. oxysporum</i> f.sp. <i>vasinfectum</i>	Israel	<i>Gossypium</i>	CBS 411.90
KSU 11404	<i>F. lateritium</i>	Australia	<i>Corylus L.</i>	Viljoen A. (Stellenbosch University, SA)
CAV 343	<i>F. oxysporum</i> f.sp. <i>melonis</i>	Israel	<i>Curcubitaceae</i>	CBS 424.90
FOG	<i>F. oxysporum</i>	Italy	<i>Orobanche ramosa</i>	Kohlschmid et al. (2009)
FT2	<i>F. oxysporum</i>	Italy	<i>Orobanche ramosa</i>	Boari et al. (2004)
AC3	<i>F. oxysporum</i>	Italy	<i>Orobanche cumana</i>	-
CAV 330	<i>F. oxysporum</i> f.sp. <i>lycopersici</i>	Israel	<i>Solanum lycopersicum</i>	CBS 413.90
FOL	<i>F. oxysporum</i>	Italy	<i>Solanum lycopersicum</i>	-
rad-lyc	<i>F. oxysporum</i> f.sp. <i>radicis-lycopersici</i>	Germany	<i>Solanum lycopersicum</i>	-
lycopers	<i>F. oxysporum</i> f.sp. <i>lycopersici</i>	Netherlands	<i>Solanum lycopersicum</i>	-

melonis	<i>F. oxysporum</i> f.sp. <i>melonis</i>	Frankreich	<i>Curcubitaceae</i>	-
gladioli	<i>F. oxysporum</i> f.sp. <i>gladioli</i>	Germany	<i>Gladiolus</i> L.	-
308	<i>F. oxysporum</i>	Germany	<i>Dianthus caryophyllus</i>	Vögele R. (University of Hohenheim, Germany)
309	<i>F. oxysporum</i>	Germany	<i>Dianthus caryophyllus</i>	Vögele R. (University of Hohenheim, Germany)
311	<i>F. oxysporum</i>	Germany	<i>Dianthus caryophyllus</i>	Vögele R. (University of Hohenheim, Germany)
312	<i>F. oxysporum</i>	Germany	<i>Dianthus caryophyllus</i>	Vögele R. (University of Hohenheim, Germany)
313	<i>F. oxysporum</i>	Germany	<i>Dianthus caryophyllus</i>	Vögele R. (University of Hohenheim, Germany)
314	<i>F. oxysporum</i>	Germany	<i>Dianthus caryophyllus</i>	Vögele R. (University of Hohenheim, Germany)
315	<i>F. oxysporum</i>	Germany	<i>Dianthus caryophyllus</i>	Vögele R. (University of Hohenheim, Germany)
316	<i>F. oxysporum</i>	Germany	<i>Dianthus caryophyllus</i>	Vögele R. (University of Hohenheim, Germany)
317	<i>F. oxysporum</i>	Germany	<i>Dianthus caryophyllus</i>	Vögele R. (University of Hohenheim, Germany)
318	<i>F. oxysporum</i>	Germany	<i>Dianthus caryophyllus</i>	Vögele R. (University of Hohenheim, Germany)
319	<i>F. oxysporum</i>	Germany	<i>Dianthus caryophyllus</i>	Vögele R. (University of Hohenheim, Germany)
320	<i>F. oxysporum</i>	Germany	<i>Dianthus caryophyllus</i>	Vögele R. (University of Hohenheim, Germany)
321	<i>F. oxysporum</i>	Germany	<i>Dianthus caryophyllus</i>	Vögele R. (University of Hohenheim, Germany)
322	<i>F. oxysporum</i>	Germany	<i>Dianthus caryophyllus</i>	Vögele R. (University of Hohenheim, Germany)
323	<i>F. oxysporum</i>	Germany	<i>Dianthus caryophyllus</i>	Vögele R. (University of Hohenheim, Germany)
324	<i>F. oxysporum</i>	Germany	<i>Dianthus caryophyllus</i>	Vögele R. (University of Hohenheim, Germany)
325	<i>F. oxysporum</i>	Germany	<i>Dianthus caryophyllus</i>	Vögele R. (University of Hohenheim, Germany)
326	<i>F. oxysporum</i>	Germany	<i>Dianthus caryophyllus</i>	Vögele R. (University of Hohenheim, Germany)
329	<i>F. oxysporum</i>	Germany	<i>Dianthus caryophyllus</i>	Vögele R. (University of Hohenheim, Germany)
W152	<i>F. oxysporum</i>	Kenya	<i>Warburgia ugandensis</i>	Mitter B. (AIT GmbH, Austria)
ACC01	<i>F. oxysporum</i>	Austria	Metal working fluid	Gorfer M. (BOKU, Austria)
ACC02	<i>F. oxysporum</i>	Austria	Metal working fluid	Gorfer M. (BOKU, Austria)
ACC03	<i>F. oxysporum</i>	Austria	Metal working fluid	Gorfer M. (BOKU, Austria)
ACC04	<i>F. oxysporum</i>	Austria	Metal working fluid	Gorfer M. (BOKU, Austria)
ACC05	<i>F. oxysporum</i>	Austria	Metal working fluid	Gorfer M. (BOKU, Austria)
ACC06	<i>F. oxysporum</i>	Austria	Metal working fluid	Gorfer M. (BOKU, Austria)
ACC07	<i>F. oxysporum</i>	Austria	Metal working fluid	Gorfer M. (BOKU, Austria)
ACC08	<i>F. oxysporum</i>	Austria	Metal working fluid	Gorfer M. (BOKU, Austria)

ACC09	<i>F. oxysporum</i>	Austria	Metal working fluid	Gorfer M. (BOKU, Austria)
NG_H15	<i>F. oxysporum</i>	Austria	Agricultural soil	Gorfer et al. (2011)
NG_H16	<i>F. oxysporum</i>	Austria	Agricultural soil	Gorfer et al. (2011)
G-5111	<i>F. nygamai</i>	Australia	Agricultural soil	Viljoen A. (Stellenbosch University, SA)
KSU 18979	<i>F. redolens</i>	Germany	Agricultural soil	Viljoen A. (Stellenbosch University, SA)
CAV 240	<i>F. oxysporum</i>	South Africa	Agricultural soil	Nel et al. (2006)
KSU 11478	<i>F. babinda</i>	Australia	Forest soil	Viljoen A. (Stellenbosch University, SA)
SC1103_05	<i>F. oxysporum</i>	Austria	Indoor air	Viljoen A. (Stellenbosch University, SA)

-.: Origin of isolate not determinable.

Table 2.

Primer	Function	Sequence
<i>Eco</i> RI-ad forward	<i>Eco</i> RI-Adapter	5'-CTCGTAGACTGCGTACC-3'
<i>Eco</i> RI-ad reverse	<i>Eco</i> RI-Adapter	3'-CATCTGACGCATGGTTAA-5'
<i>Mse</i> I-ad forward	<i>Mse</i> I-Adapter	5'-GACGATGAGTCCTGAG-3'
<i>Mse</i> I-ad reverse	<i>Mse</i> I-Adapter	3'-TACTCAGGACTCAT-5'
<i>Eco</i> RI-0	Pre-selective amplification	5'-GACTGCGTACCAATTC-3'
<i>Mse</i> I-0	Pre-selective amplification	5'-GATGAGTCCTGAGTAA-3'
<i>Eco</i> RI-ACT	Selective amplification	5'-GACTGCGTACCAATTCACT-3'
<i>Mse</i> I-AA	Selective amplification	5'-GATGAGTCCTGAGTAAAA-3'
Kb1	Fos marker	5'-GGACGAACTGACAGCCCTAC-3'
Kb2	Fos marker	5'-GTAACCGTAATATTGTTTCAGAGCTC-3'

Table 3.

Soil	Treatment	TC [g kg ⁻¹]	N _t [g kg ⁻¹]	EOC [mg kg ⁻¹]	EON [mg kg ⁻¹]	NH ₄ ⁺ [mg kg ⁻¹]	NO ₃ ⁻ [mg kg ⁻¹]	pH
Embu	C	16.9 ± 0.1a	1.51 ± 0.1ab	572 ± 9.0ab	1484 ± 55ab	3.9 ± 1.4ab	23.4 ± 2.8c	4.5 ± 0.1a
	C+S	17.2 ± 0.1a	1.43 ± 0.1a	583 ± 15.0abc	1426 ± 57a	2.8 ± 0.2a	3.9 ± 1.6a	4.7 ± 0.1a
	F	16.9 ± 0.3a	1.47 ± 0.1ab	589 ± 9.7bcd	1424 ± 12a	6.8 ± 0.3c	22.0 ± 5.9c	4.6 ± 0.1a
	F+S	17.4 ± 0.2ab	1.43 ± 0.0ab	551 ± 8.3a	1419 ± 31a	2.9 ± 0.1a	10.1 ± 1.7bc	4.8 ± 0.17a
	F+T	18.0 ± 0.5bc	1.57 ± 0.1b	619 ± 18.3cd	1556 ± 110ab	6.4 ± 0.7bc	3.6 ± 1.9a	4.9 ± 0.2b
	F+S+T	18.6 ± 0.5c	1.59 ± 0.1b	619 ± 18.2d	1581 ± 61b	5.2 ± 0.2bc	4.3 ± 2.4ab	4.9 ± 0.1b
Statistics		***	**	**	*	**	**	***
Machanga	C	3.70 ± 0.0a	0.35 ± 0.0a	103 ± 8.1a	342 ± 20a	3.6 ± 0.5a	1.99 ± 1.8a	4.7 ± 0.02a
	C+S	3.88 ± 0.2a	0.36 ± 0.0a	109 ± 5.5a	356 ± 45a	3.7 ± 1.6a	1.05 ± 0.6a	4.7 ± 0.03a
	F	3.67 ± 0.1a	0.36 ± 0.0a	100 ± 4.5a	354 ± 16a	3.5 ± 0.4a	2.07 ± 0.3a	4.7 ± 0.01a
	F+S	4.06 ± 0.2a	0.38 ± 0.0a	110 ± 16.5a	373 ± 21a	5.1 ± 1.8a	1.04 ± 0.6a	4.8 ± 0.01a
	F+T	5.64 ± 0.4b	0.57 ± 0.0b	207 ± 42.8b	557 ± 16b	15.0 ± 0.9c	3.80 ± 0.3a	5.6 ± 0.02b
	F+S+T	5.46 ± 0.6b	0.53 ± 0.0b	194 ± 41.2b	514 ± 27b	8.7 ± 1.4b	0.78 ± 0.3a	5.7 ± 0.01b
Statistics		***	**	***	**	***	n.s.	***

^aAbbreviations: TC: Total carbon, N_t: Total nitrogen, EOC: extractable organic carbon, EON: Extractable organic nitrogen, NH₄⁺: ammonium, NO₃⁻: nitrate, pH: soil pH.

^bSignificance levels: n.s., $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Development of a monitoring tool for the BCA *Fusarium oxysporum* f.sp. *strigae*

AFLP fingerprint

1. Sequencing
2. Primer development
3. Specificity testing
4. Validation in two soils

Monitoring of the BCA in soils via quantitative PCR