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Growth and colonization of organic matter in soil by *Fusarium proliferatum*

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Abstract: *Fusarium proliferatum* is a broad host range, mycotoxin-producing, seed-borne pathogen of maize. A green fluorescent, hygromycin-resistant *F. proliferatum* transformant (Fp-70–2-5-G2) was developed to provide markers to track movement and colonization of bait organic matter in non-sterile soil microcosms. Maize seeds that were heat-killed and re-colonized with Fp-70–2-5-G2 served as sources of inoculum. They were added to non-sterile soil together with heat-killed, non-colonized maize seeds (baits), at several temperatures, soil matric potentials, and source-to-bait distance combinations. Baits were retrieved from soil and plated onto Nash-Snyder medium amended with 1 µg mL^{-1} hygromycin. Fp-70–2-5-G2 grew through non-sterile sieved soil and colonized maize seed; distance, temperature and soil matric potential had significant effects ($P < 0.0001$, $P = 0.0365$ and $P < 0.0001$, respectively) on growth and colonization. The maximum distance that Fp-70–2-5-G2 grew and colonized maize seed was 1.5 cm at 25°C and −0.005 MPa matric potential. The maximum colonization of baits by Fp-70–2-5-G2 occurred at 0 cm (80%) and 0.5 cm (50%) source-to-bait distance at 25°C and −0.005 MPa matric potential. Growth through soil and colonization of bait organic matter was significantly less in soil with an intact field structure. In addition to plant and seed colonization, *F. proliferatum* may have an active soil inhabitant phase in its life cycle.

Keywords: active dispersal, *Fusarium proliferatum*, GFP transformation, hyphal growth, soil inoculum

Résumé: *Fusarium proliferatum* est un agent pathogène à large éventail d'hôtes et produisant des mycotoxines, qui est transporté par la semence de maïs. Un transformant vert fluorescent de *F. proliferatum* résistant à l'hygromycine (Fp-70-2-5-G2) a été développé pour fournir des marqueurs visant à suivre le mouvement et la colonisation de matière organique servant d'appât dans des microcosmes de sol non stérile. Les graines de maïs qui avaient été tuées par la chaleur et recolonisées par Fp-70-2-5-G2 ont servi de sources d'inoculum. Elles ont été ajoutées à du sol non stérile avec des graines de maïs tuées par la chaleur et non colonisées (appâts), et ce, à plusieurs températures, potentiels matriciels du sol et combinaisons de distances entre les sources et les appâts. Ces derniers ont été retirés du sol et placés sur un milieu de culture Nash et Snyder amendé avec 1 µg mL^{-1} d'hygromycine. Fp-70-2-5-G2 s'est développé dans un sol sassé non stérile et a colonisé les graines de maïs; la température et le potentiel matriciel du sol ont eu une influence significative ($P < 0.0001$) sur la croissance et la colonisation. La distance maximale sur laquelle Fp-70-2-5-G2 s'est développé et a colonisé les graines de maïs était de 1.5 cm à 25 °C pour un potentiel matriciel de −0.005 MPa. La colonisation maximale des appâts par Fp-70-2-5-G2 s'est produite à une distance entre la source et l'appât de 0 cm (80%) et de 0.5 cm (50%) à 25 °C pour un potentiel matriciel de −0.005 MPa. La croissance dans le sol et la colonisation de la matière organique servant d'appât étaient significativement moindres dans un sol dont la structure était intacte. En plus de coloniser les plants et les semences, *F. proliferatum* pourrait avoir, au cours de son cycle vital, une phase terricole active.

Mots clés: croissance hyphale, dispersion active, *Fusarium proliferatum*, inoculum du sol, transformation de la protéine verte fluorescente

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Introduction

Over the past few decades, the trade of food, plant and animal products has resulted in the worldwide movement, introduction and establishment of exotic pests and pathogens that in some cases has had dramatic negative impacts on native and cultivated plant systems (Jeger et al., 2011). *Fusarium proliferatum* (Matsushima) Nirenberg ex Gerlach & Nirenberg (*Gibberella fujikuroi* mating population D) is a fungal plant pathogen with a worldwide distribution. It has an extremely broad host range, colonizing and causing diseases in diverse economically important plants, including asparagus (Elmer, 1990), banana (Jimenez et al., 1993), citrus fruits (Hyun et al., 2000), onion (Stankovic et al., 2007), orchids (Benyon et al., 1996), maize (Munkvold, 2003), rice (Desjardins et al., 2000) and sorghum (Leslie, 2003). Because *F. proliferatum* can be symptomless and seed-borne in maize seeds, it has a high risk of being introduced into new environments with unknown consequences. If detected post-entry, the introduction of seed-borne organisms into new environments can disrupt trade and/or extend the geographic range of undesirable populations and/or strains e.g. high toxin-producing strains with increased aggressiveness (Eudes et al., 2001).

Fusarium proliferatum colonizes maize plants worldwide (Logrieco et al., 2002). This fungus is an effective producer of polyketide-derived fumonisin mycotoxins, with fumonisin B_1 (FB_1) being the most prevalent (Rheeder et al., 2002). Fumonisin B_1 is toxic to animals, including humans, due to inhibition of sphingolipid metabolism and cell cycle regulation. It has been associated with oesophageal cancer, liver cancer and neural tube defects in humans (Desjardins, 2006). Strains of *F. proliferatum* are reported to produce FB_1 at more than 6000 $\mu g\,g^{-1}$ (ppm) in culture (Leslie et al., 2004). In humans, there is little information on the acute toxicity of FB_1 and the LD_{50} of FB_1 is unknown. No information is available on the toxicological effects of single dose exposure to FB_1 by inhalation or dermal routes (Marasas et al., 2000). However, based on the potential adverse effects caused by fumonisins to humans and animals, the Food and Drug Administration (FDA) has made a recommendation about maximum levels of fumonisins in human food (4 ppm) and in animals feed (100 ppm) (Food and Drug Administration, 2001).

Fusarium proliferatum also produces a wide range of other mycotoxins and biologically active metabolites, including beauvericin, enniatin, fusaric acid, fusarin, fusaproliferin and moniliformin (Marasas et al., 1986; Ritieni et al., 1995; Bacon et al., 1996; Herrmann et al.,

1996; Moretti et al., 1996; Desjardins et al., 2000; Leslie et al., 2004).

Despite a global distribution and multiple hosts in diverse environments, the life cycle of *F. proliferatum* is still not well understood but it is suspected to share some similarities to that of *Fusarium verticillioides* (Battilani et al., 2003). Dispersal of seed-borne *F. proliferatum* in the field can be passive by conidia movement in water, air or by insect vectors (Munkvold, 2003). This research presents evidence that hyphal growth through soil is another means of dispersal for *F. proliferatum*.

Materials and methods

F. proliferatum *isolates*

Fusarium proliferatum was isolated from maize seed 'Pioneer 32N70' surface-sterilized with 10% bleach (containing 6% sodium hypochlorite) for 1 min, rinsed in distilled water for 30 s, and plated onto Nash-Snyder (NS) medium (Leslie & Summerell, 2006). Isolates were single-spored and grown on NS medium at 27°C for 7 days. Isolates were identified as *F. proliferatum* by morphological and molecular characteristics, including the amplification and sequencing of the *TEF-1α* and *FUM1* genes (O'Donnell et al., 1998; Stępień et al., 2011). One isolate (Fp-70–2-5) confirmed as *F. proliferatum* was used in this study.

Agrobacterium tumefaciens-*mediated transformation of isolate Fp-70–2-5*

A plasmid (pBHt2) carrying the green fluorescent protein (GFP) and antibiotic-resistance genes was used for the transformation of *F. proliferatum* following published protocols (Mullins et al., 2001; Rho et al., 2001). Transformations were considered successful when individual isolates expressed the GFP and hygromycin resistance genes. Nineteen hygromycin-resistant colonies were confirmed microscopically as green transformants and stored in 15% glycerol in cryovial tubes at −80°C.

Transformant characterization

The 19 transformed strains were tested to confirm that the insertion was mitotically stable by comparing them to the parent isolate Fp-70-2-5. Characterization included morphology, hyphal growth rate, pathogenicity on apple fruits, and colonization of maize seeds and onion tissues. The stability of the insertion was tested by sub-culturing for five generations on Spezieller Nährstoffarmer Agar (SNA) medium (Kunitake et al., 2011); the fifth generation was characterized for morphology, hyphal growth

rate, hygromycin resistance and fluorescence. Hyphal growth rate was determined by placing a colonized agar plug (~ 8 mm diameter) in the centre of NS and SNA agar medium plates and the radial growth measured daily for 7 consecutive days.

Pathogenicity was tested in apple fruits and colonization ability in maize seeds and onion bulb tissues. For pathogenicity, organically grown 'Granny Smith' apples were used; 5 puncture wounds (5 mm in diameter and 5 mm in depth) were made in each apple and 50 μL of spore suspension (1×10^6 spores mL^{-1}) was applied to the wound. Two of the wounds were used for controls – the negative control was sterile distilled water, and the positive control was the apple pathogen *Penicillium expansum*; the three other wounds were for the wild type Fp-70–2-5 and two for the transformed strain being tested. The inoculated apples were placed in a container with wet paper towels to maintain high humidity, and the container placed in an incubator at 25°C. For the maize seed colonizing test, 'Pioneer 32N70' seeds were hydrated for 5 h in distilled water and then placed in a hot water bath (65°C) for 3 min. Heat-treated seeds were subjected to one of three treatments: (1) distilled water (negative control); (2) Fp-70–2-5 (spore suspension of 1×10^6 spores mL^{-1} – positive control); or (3) a transformant strain (spore suspension of 1×10^6 spores mL^{-1}) for 20 h. Next, the seeds were surface-sterilized by soaking in 10% bleach for 1 min and rinsed in distilled water for 30 s. Finally, the seeds were plated onto NS medium and incubated at 25°C for 5 days. For the onion tissue colonization test, a thin layer of onion tissue was cut from a bulb using a sterile scalpel. The thin layer was placed on a microscope slide and subjected to one of three treatments: (1) 50 μL distilled water, (2) 50 μL Fp-70–2-5 Fp-70–2-5 (spore suspension of 1×10^6 spores mL^{-1} – positive control); or (3) 50 μL transformant strain (spore suspension of 1×10^6 spores mL^{-1}) for 48 h.

Soil collection, characterization and determination of soil water retention curve

Soil was collected from a Kansas State University experimental field in Hutchinson, KS where maize had been grown the previous season. The soil was collected from the surface horizon (~20 cm from the surface) and sieved (1.5 cm) to remove large debris. The soil characterization performed by the Kansas State University Soil Testing Lab determined a pH of 7.1, texture (72% sand, 16% silt and 12% clay), total nitrogen content (0.0901%), total carbon content (1.268%), total phosphorus content (210 ppm) and organic matter content (2.3%). The soil characterization results were consistent with the characteristics of a sandy loam Shellabarger soil series with a sand content of 35–80%, clay content of 2–19%, having a sandy loam texture with an acidic to neutral pH.

Water retention characteristics were determined experimentally at 1.5 MPa, 1.0 MPa, 0.5 MPa, 0.1 MPa, 0.033 MPa, 0.01 MPa and 0 MPa by the Kansas State University Soil Testing Lab and the Soil, Water and Plant Testing Lab at Colorado State University by using a published protocol (Klute, 1986). Similar results were obtained at both labs.

The soil water retention curve was determined using the van Genuchten model in the RETC (version 1.0) software. The van Genuchten model parameters are ideal to precisely describe a curve for a broad range of soils including disturbed and undisturbed soils (van Genuchten et al., 1991). Finally, an equation was developed to calculate the amount of water needed to add to the soil to reach the target soil matric potentials.

Source of inoculum and bait generation

Maize seeds 'Pioneer 32N70' were heat-killed in water at 75°C for 20 min and verified to be *Fusarium*-free by plating onto Nash-Snyder medium; no colonies developed. Non-viable, Fusarium-free seeds were placed in a 1×10^6 spores mL^{-1} suspension and on a rotary shaker (80 rpm) at 25°C for 16 h to allow colonization by: (i) wild-type *F. proliferatum* Fp-70–2-5 (hygromycin-sensitive, non-fluorescent) or (ii) *F. proliferatum* Fp-70–2-5-G2 (hygromycin-resistant, green fluorescent); (iii) non-viable, Fusarium-free seeds placed in sterile water served as the negative control. *Fusarium proliferatum*-colonized seeds were surface-sterilized for 1 min and rinsed in distilled water (30 s). These *F. proliferatum*-colonized seeds served as the sources of inoculum. Non-colonized, non-viable seeds were surface-sterilized as previously and rinsed with distilled water (30 s). These non-colonized, non-viable seeds were used as bait organic matter.

Colonization of baits and statistical analyses

Percentage colonization of baits was determined as a function of temperature (10°C, 25°C and 35°C), soil matric potentials (−0.005 MPa, −0.015 MPa, −0.033 MPa and −0.1 MPa), and source-to-bait distance in soil (0 cm, 0.5 cm, 1 cm and 1.5 cm). Five replicates were used for each temperature–soil matric potential–distance combination and the experiment was conducted twice.

Preliminary data demonstrated that *F. proliferatum* hyphae did not grow more than 2 cm from the source of inoculum and that the optimum sampling times were 5 days for 0 cm, 7 days for 0.5 cm, 10 days for 1.0 cm and 14 days for 1.5 cm. At the appropriate sampling times, the sources of inoculum were retrieved from soil, surface sterilized, rinsed in distilled water for 30 s, plated onto NS agar amended with hygromycin (1 µg mL^{-1}), and incubated at 27°C.

The experiment was analysed as a split plot, with temperature (10°C, 25°C or 35°C) as the whole plot treatment and each distance–soil matric potential combination as the subplot treatment. A total of 48 combinations (3 temperatures × 4 distances × 4 soil matric potentials = 48) were tested. Soil matric potential values were linearized by transformation to natural logarithms. The data were analysed using SAS® PROC MIXED (Version 9.3; SAS Institute Inc., Cary, NC), with experiment as a random effect and distance and soil matric potential modelled as covariates.

To better mimic field conditions, soil columns with an intact soil structure were obtained using a golf cup cutter. Small wells were sunk into the soil columns to a depth of 2 cm; baits were placed into wells at 0 cm, 0.5 cm, 1 cm or 1.5 cm from the source well and covered with soil. Constant temperature (25°C) and soil moisture conditions (−0.005 MPa) were used. Five replicate wells were used for each distance and the experiment was repeated twice.

Results

Characterization of transformed strain

One transformant (Fp-70–2-5-G2) with strong fluorescence (GFP) and stable hygromycin resistance was selected for further characterization (Fig. 1a). With respect to morphology and pathogenicity, the transformed strain and the wild-type strain were identical. Morphologically, the transformed strain Fp-70-2-5-G2 and the wild type Fp-70-2-5 were identical: macroconidia were slender and 3- to 5-
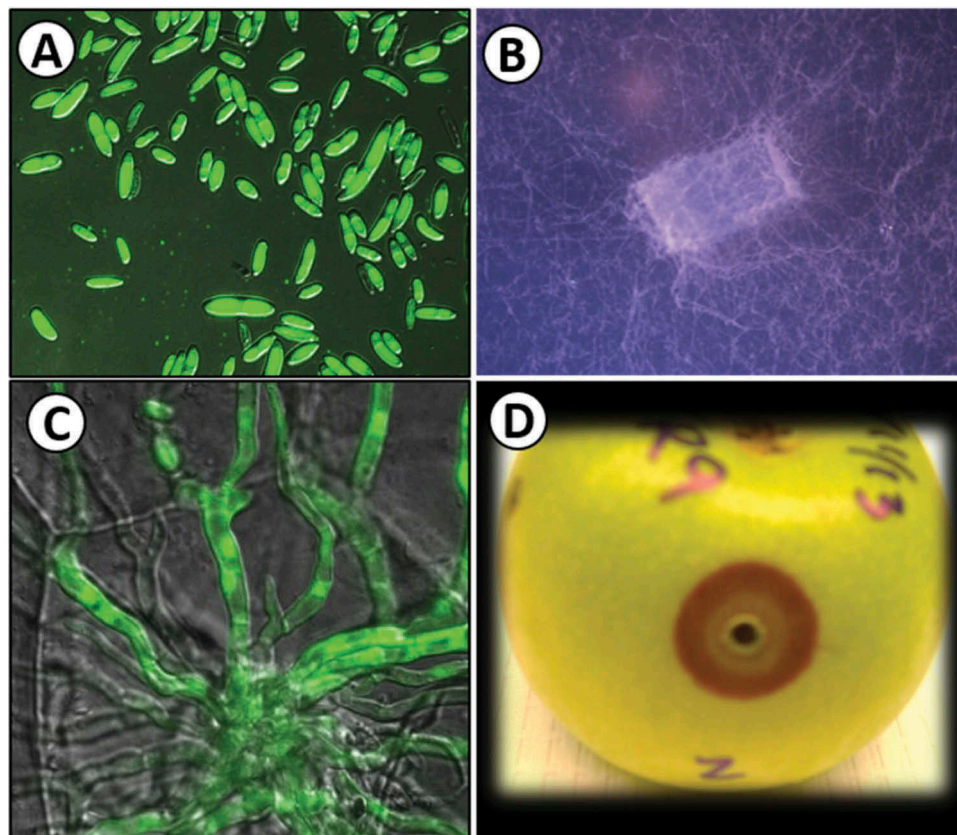

Fig. 1 (Colour online) The transformed strain Fp-70–2-5-G2 was stable and identical to the parent strain Fp-70–2-5 in growth rate and colonization of plant tissues; (**a**) 100% of conidia expressed the GFP and hygromycin-resistance traits, (**b**) growth on agar media was normal, (**c**) Fp-70–2-5-G2 readily colonized onion tissues and (**d**) Fp-70–2-5-G2 was pathogenic to apple.

septate, microconidia were club shaped and formed in chains from mono- and poly-phialides (Leslie & Summerell, 2006). Radial hyphal growth rate for the transformed strain and the wild type were indistinguishable on the two media, SNA ($R^2 = 0.99880$) and NS ($R^2 = 0.99661$) (Fig. 2).

After five successive generations, the morphology of the transformed strain Fp-70–2-5-G2 was normal (Fig. 1b) and remained identical to the wild type Fp-70-2-5 with no change from the first generation; the radial hyphal growth rate remained indistinguishable on the two media, NS ($R^2 = 0.99606$) and SNA ($R^2 = 0.99830$) (Supplementary Table 1). After five successive generations, there were no changes in fluorescence or hygromycin-resistance: the transformation was stable (Supplementary Table 1).

Colonization of maize seeds and onion tissues (Fig. 1c) and pathogenicity to apples (Fig. 1d) by the transformed strain was identical to the wild type. After five successive generations, there were no changes in pathogenicity to either apples or maize seeds. Both the transformant and wild type developed lesions on apples which had 3.6 cm diameter on average 12 days after inoculation (Fig. 1d). Maize seeds were 100% colonized by both the transformant and the wild type.

Colonization of baits at different temperatures, distances and soil matric potentials

The results of the analysis of covariance are reported in Table 1. The colonization of the baits was significantly affected by temperature ($P = 0.0365$) and was linearly related to both distance ($P < 0.0001$) and soil matric
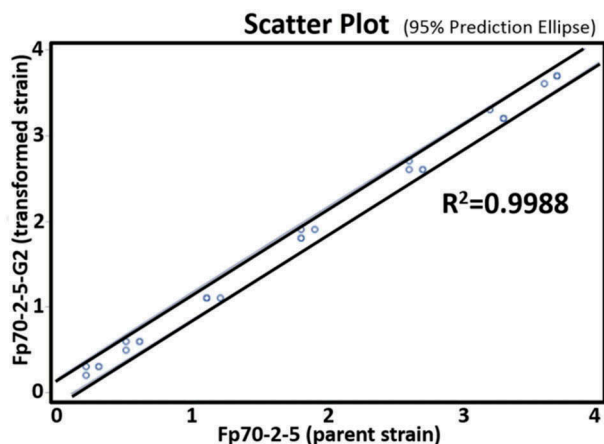

Fig. 2 Radial hyphal growth of the transformed strain Fp-70–2-5G2 and the wild type Fp-70–2-5 were not significantly different on synthetic nutrient agar ($R^2 = 0.9988$).

Table 1. Effect of temperature, distance and soil matric potential and their interactions on hyphal growth and colonization of baits by *Fusarium proliferatum* strain Fp-70–2-5-G2.

Distance (cm)	Soil Matric Potential (MPa)	Colonization (%)[a]		
		10°C	25°C	35°C
0	-0.005	40	80	50
0	−0.015	10	30	10
0	−0.033	0	20	0
0	−0.1	0	10	0
0.5	-0.005	20	50	30
0.5	−0.015	0	30	10
0.5	−0.033	0	0	0
0.5	−0.1	0	0	0
1	-0.005	0	30	0
1	−0.015	0	10	0
1	−0.033	0	0	0
1	−0.1	0	0	0
1.5	-0.005	0	10	0
1.5	−0.015	0	0	0
1.5	−0.033	0	0	0
1.5	−0.1	0	0	0

Individual Effects and Interactions of Variables	P-value
Temperature	0.0365
Distance	< 0.0001
Distance × Temperature	0.1802
Ln(Soil Matric Potential)	< 0.0001
Ln(Soil Matric Potential) × Temperature	0.0015
Distance × Ln(Soil Matric Potential)	< 0.0001
Distance × Ln(Soil Matric Potential)*Temperature	0.4789

[a] Values represent the mean percentage colonization of organic matter baits.

potential ($P < 0.0001$). There was a linear decrease in colonization of baits with increasing distances between source of inoculum and baits and a similar trend occurred with decreasing soil matric potentials. The interaction of soil matric potential and temperature ($P = 0.0015$) and the interaction of distance and soil matric potential ($P < 0.0001$) were also significant, indicating that the slopes associated with soil matric potential varied with both temperature and distance (Tables 1 and 2). To illustrate these significant interactions, the covariate models for bait colonization at each temperature, soil matric potential, and distance combination are graphically depicted in Fig. 3. The covariate models accounted for 78.8% of the variation in the data set (Fig. 4), which validates the fitness of the models to explain the differences in colonization for the different temperatures, distances and soil matric potentials.

The slopes for soil matric potential had the greatest spread at smaller distances for the three temperatures. At distances greater than 1 cm, the interaction of soil matric potential and distance produced convergence of the lines across all temperatures. The average decline in % colonization with distance

Table 2. Slope and intercept estimates from covariate analysis of distance (D) and soil matric potential (SMP) effects on percentage colonization of baits by *Fusarium proliferatum* strain Fp-70–2-5-G2.

		Slope estimates		
Temperature (°C)	Intercept	Distance	Ln (SMP)	D × Ln (SMP)
10	74.55 b[a]	−58.37 a	−11.80 b	9.22 a
25	159.95 a	−93.42 a	−23.48 a	13.30 a
35	98.63 b	−75.25 a	−15.56 b	11.88 a

[a]Intercepts and slopes within a column with the same letter are not different based on a pairwise t-test (P = 0.05).

was similar for all temperatures which reflected the similarity (non-significant interaction of distance and temperature, P = 0.1802) in slopes for distance. Finally, the soil matric potential slopes were shifted upward when temperature was 25°C which reflects the significant effect of temperature on hyphal growth through soil, i.e. under the same soil matric potential and source-to-bait distances, more baits were colonized at 25°C than at 10°C or 35°C. The models demonstrate that, at distances up to 1 cm and soil matric potentials up to −0.033 MPa, the

colonization of baits decreased in a linear fashion as either the soil matric potential decreased or the distance between the source of inoculum and the bait increased (Fig. 3). Beyond a distance of 1 cm or soil matric potential of −0.033 MPa, estimated % colonization was generally not different from zero (P > 0.05).

Sieving soil alters its natural structure, which could influence the hyphal growth and colonization of baits. Therefore, to determine if colonization could occur in soil with an intact field structure, soil columns were obtained using a golf cup cutter. The experiment was conducted at −0.005 MPa and 25°C, and was repeated once. In the first experiment, *F. proliferatum* colonized 80% of the baits at 0 cm and 20% of the baits at 0.5 cm. In the second experiment *F. proliferatum* colonized 60% of the baits at 0 cm and 40% of the baits at 0.5 cm.

Discussion

In this study, *F. proliferatum* grew through non-sterile soil (sieved reconstituted soil and intact soil columns) and colonized bait organic matter. Optimal edaphic
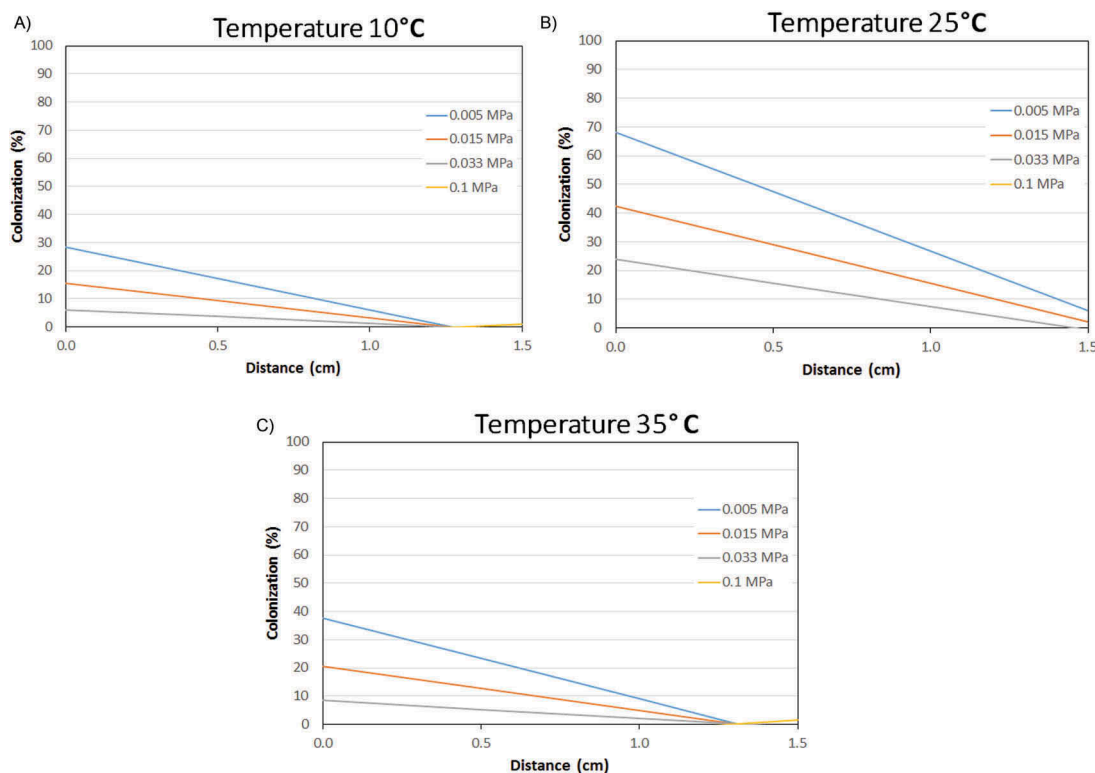

Fig. 3 (Colour online) Growth and colonization of organic matter baits in a non-sterile soil microcosm by *Fusarium proliferatum* strain Fp-70–2-5-G2 as a function of soil water potential and temperature. Covariate models for bait colonization vs. distance, log-transformed soil matric potential (SMP), and the distance × SMP interaction are presented for each of three temperatures; (A) 10°C, (B) 25°C and (C) 35°C. Estimates for SMP of −0.1 MPa were not different from zero (P > 0.05).
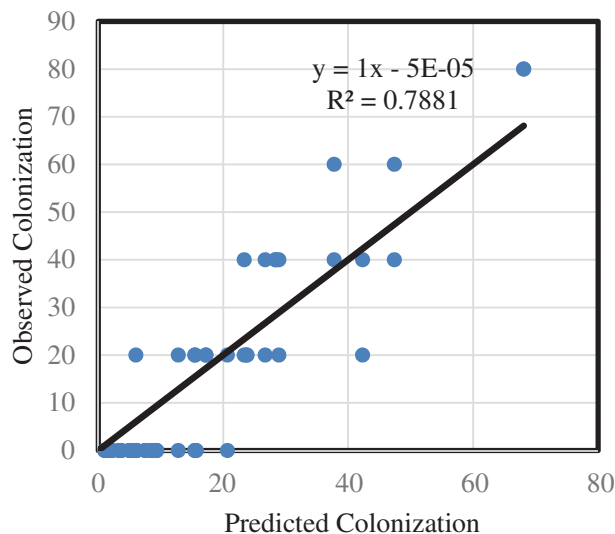
Fig. 4 (Colour online) Predicted and observed colonization of organic matter baits by *Fusarium proliferatum* strain Fp-70–2-5-G2. Covariance model fit for bait colonization data. Predicted colonization was based on linear models of bait colonization vs. distance, log-transformed soil matric potential (SMP), and the distance × SMP interaction for each of three temperatures.

conditions for hyphal growth and colonization of bait organic matter were 25°C and −0.005 MPa. The colonization of baits decreased as the distance between the source of inoculum and the bait increased. The baits used in this experiment were non-viable heat-treated maize seeds free of seed-borne organisms. In natural field soils, organic matter is more diverse with respect to chemical composition and microbial communities which may influence the colonization potential of *F. proliferatum*. However, *F. proliferatum* has a wide host range that includes monocots and dicots and is a successful saprophyte (Cotten & Munkvold, 1998) which makes it fit to compete for available organic matter with other microorganisms.

Many organisms are associated with the soil microbial community including fungi, bacteria and nematodes (Neher & Campbell, 1994; Fierer et al., 2005) that may compete with *F. proliferatum* for available organic matter. A previous study performed *in vitro* found that different fungi including *Aspergillus flavus*, *A. niger*, *A. ochraceus* and *Penicillium implicatum* inhibited colonization of maize grain by *F. proliferatum* (Marin et al., 1998a). However, additional research demonstrated that *F. proliferatum* is very competitive and dominant against *Penicillium* spp. and *A. flavus* when competing for a maize seed, at least in culture (Marin et al., 1998b). In our experiments, to simulate field conditions of competition, these experiments were performed in non-sterile soil microcosms. The ability of *F. proliferatum* to grow by hyphae and colonize organic matter under different temperatures and soil matric potentials may provide an advantage in the competition for available resources in soil with organisms more susceptible to different environmental conditions.

There is evidence that *F. proliferatum* can survive in soil for extended periods of time (Logrieco & Bottalico, 1988; Leslie et al., 1990; Logrieco et al., 1995), at least 630 days on the soil surface or in buried maize residue; colonized residue can act as a source of inoculum (Cotten & Munkvold, 1998). For this reason, ears of maize which have seeds colonized by *F. proliferatum* could also serve as maize residue in the field which can help it get introduced and dispersed. The colonization of organic matter in soil from the sources of inoculum can enhance the survival capacity of *F. proliferatum* in the field which can help it get established in new environments. In addition, the colonization of organic matter in the field, suggests that this fungus may have an active soil inhabitant phase in its life history. Furthermore, during different stages of fieldwork (e.g. tillage, sowing, harvest, etc.), a vast amount of organic matter is incorporated into soil which greatly increases the available substrate for utilization by *F. proliferatum*.

These characteristics, including the presence of ears as maize residue in the field and the different stages of fieldwork, increase the risk of *F. proliferatum* being introduced into new environments by maize seed. This could result in high-consequence strains of this fungus (i.e. high toxin producers, aggressive strains) becoming invasive in these new environments where they could affect native and cultivated plant species as well as the native fauna. Since maize is the most extensively produced agricultural commodity in the USA (Food and Agriculture Organization, 2011) and is regularly traded with other countries (import and export), there is a high probability that new strains are being introduced into new environments where they can establish (Elmer, 1995).

A previous study investigated the effects of water activity (a_w), pH and temperature on growth of *F. proliferatum* isolates from maize and reported that growth was optimum at 0.994–0.90 a_w in the temperature range of 20–35°C (optimum of 25°C) and pH of 5.5. Growth was also reported to occur at 4°C and 0.994–0.96 a_w but no growth was recorded at 40 and 45°C even under ideal a_w (Marin et al., 1995). Another study showed that germination of microconidia of *F. proliferatum* was optimal at 30°C (Marin et al., 1996). These studies were conducted *in vitro* in artificial systems. There

were no known reports about the hyphal growth of *F. proliferatum* in non-sterile soil under different temperature and soil matric potential combinations.

Fusarium proliferatum was capable of growing through sieved-soil microcosms and intact soil columns collected from an agricultural field. The distance that hyphae grew through soil and the proportion of baits colonized was much lower in soil with the intact structure. This may have been the result of a more restrictive pore size distribution increasing the difficulty for hyphal growth and the natural physical barriers present in soil (Christensen, 2001).

This study demonstrated that hyphal growth through soil can be a means of short-range active dispersal for *F. proliferatum* and might suggest that it can have an active soil inhabitant phase in its life history.

Supplymentary material

Supplemental data for this article can be accessed online here: https://doi.org/10.1080/07060661.2018.1522374.

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