INFECTION AND COLONIZATION OF TURF-TYPE BERMUDAGRASS BY *OPHIOSPHAERELLA HERPOTRICHA* EXPRESSING GREEN (GFP) OR RED (tdTOM) FLUORESCENT PROTEINS

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2002

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2008

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ACKNOWLEDGEMENTS

I would like to thank my major adviser, Dr. Nathan R. Walker, for his patience and guidance throughout the duration of this study. It is a great honor to work with him and be part of this project. My sincere appreciation is due to my committee members Dr. Stephen M. Marek, and Dr. Thomas K. Mitchell for their critical evaluations, helpful suggestions, and guidance during the course of this program. I also thank the United States Golf Association for funding this project.

I am also sincerely acknowledging the financial and technical support I received from the Department of Entomology and Plant Pathology. I thank my fellow graduate students Madhavi Dhulipala and Ian Moncrief, our student assistant Ashleigh Grace, and our research specialist Kelli Black for their technical assistance and friendship. I also thank all the faculty and staff in the Department for their support and assistance.

I also sincerely appreciate my sponsors, the OSU Office of International Students and Scholars (ISS), the Institute of International Education (IIE), the United States Department of State through Fulbright Program, and the Philippine-American Educational Foundation for their tremendous support and guidance.

My special gratitude is expressed to my parents Rolly and Leah, and in-laws Danilo and Rosemarie, for their moral support and encouragement. My utmost gratitude is expressed to my wife Donna and daughter Danielle for their love, care, and support. And finally, I praise and thank my God Lord Jesus for his guidance and gift of wisdom.

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CHAPTER I

INTRODUCTION

Bermudagrass as Turfgrass. Bermudagrass, which is thought to have originated in the Indo-African region, is now widely distributed throughout the tropical and subtropical areas around the world (Jones, 1985; McCarty and Miller, 2002). It is believed to have been introduced into the United States from the Caribbean Islands and Central America in the mid-1700's (Kneebone, 1966). In the early 20th century in North America, bermudagrass was mainly used as forage grass for livestock (Fields, 1902). Since then, many new cultivars have been developed and have found such uses as groundcover for barren lands to a superior turfgrass for urban utilization (Long, 1972). Currently, it is used for turfgrass in many lawns, open spaces, athletic fields, and golf courses (McCarty and Miller, 2002).

Bermudagrass (*Cynodon* sp. (L.) Rich) is a vigorous, stoloniferous, and rhizomatous perennial member of the family Gramineae (Poaceae) or the grass family (Jones, 1985; Taliaferro, 2003). Bermudagrass is considered one of the most important turfgrasses in North America (Casler and Duncan, 2003; Taliaferro, 2003). The two most common species used for turf in the genus *Cynodon* (L.) Rich are common bermudagrass (*Cynodon dactylon* (L.) Pers.) and interspecific hybrids of *C. dactylon* and African bermudagrass (*C. transvaalensis* Burtt-Davy) which can be dwarf, sterile and/or vegetatively-propagated (Taliaferro, 2003).

As described by Taliaferro (2003), bermudagrass has glabrous or waxy culm with nodes and hollow culm intenodes which compose the stem. The horizontal stems are called stolons (above ground) and rhizomes (underground) (Berg, 1972). These organs are important in vegetative growth and reproduction. Plants spread through creeping stolons and soil penetrating rhizomes on which new shoots and roots emerge (Taliaferro, 2003). Bermudagrass is a C_4 grass and requires high temperatures for optimal photosynthesis and is generally regarded as heat and drought tolerant although it has low tolerance to freezing temperatures (Jones, 1985; Taliaferro, 2003). It requires 29 to 38°C air temperatures and 27 to 35°C soil temperatures for optimal growth. Temperatures reaching 49°C or higher are lethal to the grass whereas temperatures below 10°C may induce dormancy or browning (McCarty and Miller, 2002).

Hybrids of bermudagrass are products of crosses between the two different species of the *Cynodon* genera. Interspecific hybridization and introduction of mutations have been used in breeding Fl hybrid clonal cultivars. With a base chromosome number of 9, the common bermudagrass *C. dactylon* var. *dactylon* has 36 chromosomes (tetraploid) whereas African bermudagrass (*C. transvaalensis*) has 18 (diploid) (Taliaferro, 2003). African bermudagrass is endemic to the Transvaal Valley of South Africa and was introduced into U.S. in the 1920's and has been used in interspecific hybridizations with *C. dactylon var. dactylon* to produce turf-type cultivars (Taliaferro, 2003; Wu et al., 2005). Utilizing AFLP markers, previous studies have revealed that *C. transvaalensis* has a genetically diverse background which has been exploited for its cold hardiness, fine texture, and low mowing height qualities (Wu et al., 2005). Crosses

between *C. dactylon var. dactylon* and *C. transvaalensis* have produced the well known sterile triploid cultivars with Tifway 419, Tifgreen and Midlawn (Taliaferro, 2003).

According to Burton (1966), Tifway is a chance hybrid between *Cynodon dactylon* and *C. transvaalensis* with a chromosome number 2n = 27. It is very dark green in color and requires less nitrogen. Tifway is a sterile cultivar and well adapted to southern states. It is less winter hardy than Tifgreen and extremely susceptible to the disease spring dead spot (Crahay et al., 1988).

Pair et al. (1994) described the characteristics of 'Midlawn' turf bermudagrass (*Cynodon dactylon* (L.) Pers. X *Cynodon transvaalensis* Burtt-Davy). Midlawn was developed by the Kansas Agricultural Experiment Station and released by the Kansas and Oklahoma Agricultural Experiment Stations in 1991. It is sterile and has high tolerance to cold. It has high overall turf quality, is slow growing, and less aggressive compared to other cultivars but has finer texture. It is recommended for use in the “transition zone” of the USA, the region where bermudagrass enters dormancy during the winter, where a high quality turf is desired, including golf courses, home lawns, and commercial turf areas. It has good resistance to spring dead spot disease (SDS) caused by *O. herpotricha* (Fr.:Fr.) J.C. Walker and has better green up traits compared to other cultivars (Baird et al., 1998). Greenhouse evaluation of cultivars revealed that root discoloration of Midlawn inoculated with *O. herpotricha* was extensive but root weight reduction was less compared to other cultivars (Baird et al., 1998).

In a study conducted in Oklahoma, Jackpot, Poco Verde, Guymon, and Common were found to be susceptible to spring dead spot (Baird et al., 1998). African bermudagrass, Guymon, Sundevil, Midlawn, Midfield, Ft. Reno, Mirage, and several

experimental seed-propagated entries, on other hand, were found to be more resistant to SDS (Baird et al., 1998).

Spring Dead Spot. Spring dead spot (SDS) caused by *Ophiosphaerella* spp. is the most important disease of *Cynodon dactylon* (L.) Pers. and its hybrids in North America and Australia (Smith, 1971; Lucas, 1980; Smiley et al., 2005; Taliaferro et al., 2004). Bermudagrass cultivars that are used as turf for home and institutional lawns, athletic fields, parks and golf courses in the transition zone are commonly affected.

In 1960, Wadsworth and Young reported the prevalence of this disease in Oklahoma. The disease was observed in a bermudagrass lawn at Stillwater, Oklahoma during the spring of 1954 but was reported to have been observed since 1936. Before 1960, a disease similar to SDS was reported in other states such as Kansas, Nebraska, Pennsylvannia, Missouri, and Arkansas (Wadsworth and Young, 1960). In Australia, spring dead spot on bermudagrass was first reported in 1961 by Smith (1965).

Smiley et al. (2005) described the symptoms as circular patches of bleached, dead grass that appear in the spring as the dormant grass resumes growth. Typically, symptoms appear after the stand is three years old and when the quality of maintenance and management is usually high (Lucas, 1980; Smiley et al., 2005; McCarty and Miller, 2002). The margins are usually even but may become irregular when spots have coalesced to form large, dead areas up to a meter in diameter. Patches may become doughnut-shaped or ring-like as the grass recovers and grass in the center is no longer affected by the disease (Wadsworth and Young, 1960; Smiley et al., 2005). Foliage of the dead grass appears bleached or straw colored. Decayed roots and stolons in affected areas are common as infected roots are invaded by microorganisms. Plants that survive are

generally stunted. (Smiley et al., 2005). The dead patches create aesthetic problems for most all bermudagrass applications. The disease is especially problematic for golf courses due to the appearance of symptoms in spring and early summer when tournaments are common and rehabilitation of the affected areas is challenging.

Smiley et al. (2005) described the fungi that cause SDS as having dark brown, septate mycelia with sclerotia produced on infected roots and stolons. They also described ascocarps such as pseudothecia on dead tissues. Crahay et al. (1988) described the pseudothecia on inoculated roots as black, flask-shaped, with a thick neck. However, they did not observe sclerotia or sclerotia-like bodies in their study.

Disease Management. Proper cultural practices may reduce the frequency and severity of SDS. For instance, a combination of aeration plus vertical mowing, performed twice a year, was reported to reduce the severity of SDS (Tisserat and Fry, 1997). Fungicides containing the active ingredients such as myclobutanil, propiconazole, diniconazole, fenarimol and benomyl have provided excellent control of SDS (Ohr et al., 1987). Likewise, fungicides containing triadimenol and penconazole can provide some control of SDS (Ohr et al., 1987). Ammonium-based nitrogen fertilizers with potassium are also recommended as this combination can improve spring green up and reduce the severity of SDS (Dernoeden et al., 1991). Winter-hardy cultivars typically are more tolerant of SDS (Smiley et al., 2005).

The Causal Agent. Three fungal species are known to incite spring dead spot. These are: *Ophiosphaerella herpotricha* (Fr.) Walker; *Ophiosphaerella korrae* (J. Walker and A.M. Smith) R.A. Shoemaker and C.E. Babcock; and *Ophiosphaerella narmari* (J. Walker and A.M. Smith) Wetzel, Hubert, and Tisserat (Smith, 1965; Endo et al., 1985;

Tisserat et al., 1989; Wetzel et al., 1999a; Smiley, 2005). *Ophiosphaerella herpotricha* is considered the primary SDS causal agent in the central plains of North America (Smiley et al., 2005, Wetzel et al., 1999a). *Ophiosphaerella korrae* is the principal causal agent in East, West, and Gulf Coast regions of the U.S. (Crahay et al., 1988; Endo et al., 1985). However, it has been isolated in several locations such as Oklahoma, Kansas, and Kentucky (Iriarte et al., 2004; Wetzel et al., 1999b). *Ophiosphaerella narmari* has been reported in Australia (Hawkes, 1987) and was later reported to be present in North America (Wetzel, et al., 1999b, Iriarte et al., 2004).

The causal agent of spring dead spot was unknown until 1965 when Smith (1965) demonstrated that the disease was caused by *Ophiobolus herpotrichus* but later renamed it *Leptosphaeria korrae* J. Walker & A. M. Smith. Smith (1971) later confirmed that SDS in New South Wales was caused by the fungus *Leptosphaeria narmari* but did not eliminate *L. korrae* as a possible causal agent, as *L. korrae* occasionally caused similar symptoms on couch grass (*Cynodon dactylon* (L.) Pers.). In the United States, Endo et al. (1985) demonstrated that *Leptosphaeria korrae* is the causal agent of SDS disease of bermudagrass in California. *Ophiobolus herpotrichus* was renamed later to *Ophiosphaerella herpotricha* (Fr.) Walker whereas *Leptosphaeria korrae* and *Leptosphaeria narmari* were renamed *Ophiosphaerella korrae* and *Ophiosphaerella narmari*, respectively (Wetzel et al., 1999b).

The taxonomic placement of *Ophiosphaerella* spp. is:

Domain: Eukarya

Kingdom: Fungi

Phylum: Ascomycota

Class: Dothideomycetes

Order: Dothideales

Genus: *Ophiosphaerella*

Species: *Ophiosphaerella herpotricha*

Ophiosphaerella narmari

Ophiosphaerella korrae

Ophiosphaerella agrostis

Ophiosphaerella herpotricha is sterile in culture with mycelium containing septa that delineate cells (Tisserat, 1989). However, according to Smiley et al. (2005), these fungi can produce sexual spores (ascospores) within an ascus in pseudothecia.

Infection and Colonization of Bermudagrass Roots. Based on ecological groupings, *Ophiosphaerella* species are facultative saprophytes particularly associated with the plant root system (Smiley et al., 2005). Colonization and infection of roots by these ectotrophic root-infecting fungi may occur when soil temperatures ranging from 10 to 25°C while maximum injury may occur at 15°C (Smiley et al., 2005). Root lesions

caused by colonization by *O. herpotricha* were greatest at 17°C and minimal at 25°C for studies conducted in environmental chambers (Walker et al., 2006)

SDS Diagnosis. The causal agents of SDS have been identified by visual observation using artificial media culture techniques and microscopy. *Ophiosphaerella* spp. can be grown on culture media such as potato dextrose agar and malt extract agar (Clarke and Gould, 1993). *Ophiosphaerella herpotricha* initially produces white, cottony mycelium on these agar media. Cultures turn tan to brown in 3 to 7 days and later turned darker. *Ophiosphaerella narmari* and *O. korrae*, on the other hand, may produce white mycelia at first and then turn darker or black at maturity. Optimal growth in culture is achieved between 20 to 25 °C (Tisserat et al., 1989). Pseudothecia may be observed and vary in color and shape depending on the species. Asci and ascospores are also described and used to identify the species (Clarke and Gould, 1993). However, sole identification of these SDS fungi using the differences in their microscopic and cultural characteristics is sometimes unreliable.

Recent developments in biotechnology have paved the way to better identification and detection of SDS fungi. Universal primers such as ITS4 and ITS5 have been used to amplify the internal transcribed spacer (ITS) region of ribosomal DNA of *O. herpotricha* and *O. korrae* using polymerase chain reaction (Tisserat et al., 1994). Specific primers, OHITS1 and OHITS2 for *O. herpotricha* and OKITS1 and OKITS2 for *O. korrae* were developed by Tisserat et al. (1994) to differentiate isolates. In a phylogenetic study, all *Ophiosphaerella* species were found to have high levels of sequence similarity, but distinct from other genera of ectotrophic root-inhabiting fungal species (Wetzel et al., 1999b).

Host-Pathogen Interaction. Studies to elucidate the interaction between the *Ophiosphaerella* and bermudagrass at the cellular level have been conducted but with little success (Smiley et al., 2005). Studies by Endo et al. (1985) found hyphae and a brown occluding substance occurring within the xylem vessels of roots infected by *O. korrae*. Sclerotia were also found on the root surface and within the cortex of infected roots. Infection of the stele was noted and occurred later in disease development. Small brown flecks that turn into black or brown dry rot or necrotic tissues were observed on culm bases, rhizomes, stolons, and roots. A similar study conducted by Walker et al. (2005) showed that Tifway cultivar roots inoculated with *O. herpotricha* exhibited brown to tan discoloration and the fungus could colonize large areas of the roots. Baird et al. (1998) reported root discoloration for cultivars including the resistant cultivar Midlawn and susceptible Tifway when inoculated with *O. herpotricha*.

The biology and ecology of these fungi are not yet completely understood since the disease was first described more than 50 years ago (Endo et al., 1985; Crahay et al., 1988; Tisserat et al., 1989). A better understanding of the etiology and host-plant interactions of SDS will generate information that can be used to improve disease resistance of cultivars through traditional breeding or genetic engineering efforts. Although temperature has been identified as an important factor that influences the disease, little is known about the effect of this factor or other factors on the interaction between the host and *Ophiosphaerella* at the cellular level (Tisserat et al., 1989, Baird et al., 1998; Walker et al., 2006).

The objectives of this research were to: i) transform isolates of *Ophiosphaerella herpotricha* to express green (GFP) and red (tdTom) fluorescent proteins; ii) describe

infection and colonization of the roots of three turf-type bermudagrasses, Tifway 419, Midlawn, and *C. transvaalensis* by fluorescent protein expressing isolates of *O. herpotricha*; and iii) describe infection and colonization of bermudagrass stolons by fluorescent protein expressing isolates of *O. herpotricha*.

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CHAPTER II

INFECTION AND COLONIZATION OF THE ROOTS OF THREE TURF-TYPE BERMUDAGRASSES BY *OPHIOSPHAERELLA HERPOTRICHA* EXPRESSING FLUORESCENT PROTEINS

Introduction

Spring dead spot (SDS) caused by *Ophiosphaerella herpotricha* (Fr.) Walker; *O. korrae* (J. Walker and A.M. Smith) R.A. Shoemaker and C.E. Babcock; and *O. narmari* (J. Walker and A.M. Smith) Wetzel, Hubert, and Tisserat (Smith, 1965; Endo et al., 1985; Tisserat et al., 1989; Wetzel et al., 1999b; Smiley, 2005) is the most damaging and important disease of turf-type bermudagrass species (*Cynodon dactylon* (L.) Pers.) and hybrid cultivars (*Cynodon dactylon* x *C. transvaalensis* Burtt-Davy) in North America and Australia (Smith, 1971; Lucas, 1980; Taliaferro et al., 2004; Smiley et al., 2005). Bermudagrass cultivars that are used as turf for home and institutional lawns, athletic fields, parks, and golf courses in regions where grass is dormant during the winter are commonly damaged by SDS. The disease is often most severe where the stand is three years old or older, the turf maintenance and management is intensive, and normal winter dormancy is artificially delayed (Lucas, 1980; McCarty and Miller, 2002; Smiley et al., 2005).

Ophiosphaerella herpotricha is considered the primary SDS causal agent in the midwestern region of North America (Wetzel et al., 1999a; Smiley et al., 2005). The biology and ecology of these fungi are not yet completely understood since the disease was described more than 50 years ago (Endo et al., 1985; Crahay et al., 1988; Tisserat et al., 1989). Although temperature has been identified as an important factor that influences the disease, little is known about how this factor and other factors affect the interaction of the host and the fungus at cellular level (Tisserat et al., 1989, Baird et al., 1998; Walker et al., 2006). Smiley et al. (2005) suggested that soil temperatures ranging 10 to 25°C favor colonization and infection of roots by *O. herpotricha* and *O. korrae* and a temperature of 15°C favors maximum injury. Walker et al. (2006) found root lesion caused by colonization by *O. herpotricha* was greatest at 17°C and minimal at 25°C when grown under controlled environment conditions.

Colonization by *O. herpotricha* directly affects the roots and stolons of the grass and results in discoloration and necrosis (Smith, 1965; Tisserat et al., 1989; Smiley et al., 2005). Smiley et al. (2005) described the fungi that cause SDS as having dark brown, septate mycelia that produce sclerotia on infected roots and stolons. They described hyphal growth on roots and stolons to be similar to that of take-all pathogen *Gaeumannomyces graminis* var. *avenae*. According to Smiley et al. (2005) finer hyphae infect the root and fuse to form large, dark, oblong infection mats, 60 to 400 µm in diameter. The presence of ascocarps such as pseudothecia or perithecia which are black and thick-walled on roots, stolons or leaf sheaths was also noted. Crahay et al. (1988) described pseudothecia on inoculated roots as black, flask-shaped, with a thick neck. However, they did not observe sclerotia or sclerotia-like bodies. Studies by Endo et al.

(1985) found hyphae and a brown occluding substance of *O. korrae* occurring within the xylem vessels of the roots. Sclerotia were also found on the root surface and within the cortex of infected roots. Infection of the stele was observed and occurred later in disease development. Small brown flecks that turn into black or brown dry rot or necrotic tissues were observed on culm bases, rhizomes, stolons and roots. A similar study conducted by Walker et al. (2005) showed that Tifway cultivar roots inoculated with *O. herpotricha* exhibited brown to tan discoloration and the fungus colonized large areas of the roots. Hybrid cultivars that are more tolerant to winter-injury are often less susceptible to SDS (Tisserat et al., 1989, Baird et al., 1998). Baird et al. (1998) reported root discoloration for both the resistant cultivar Midlawn and susceptible Tifway when inoculated with *O. herpotricha*.

Utilization of fluorescent protein expression in fungi introduced through *Agrobacterium tumefaciens*-mediated transformation has greatly aided the study and understanding of disease progression *in planta*. This technique has also allowed for the visualization and analysis of colonization and infection of plant pathogens *in vivo* (Lorang et al., 2001; Lagopodi et al., 2002; Sesma and Osbourn, 2004). The green fluorescent protein (GFP) has been used as a reporter gene in many organisms including bacteria, viruses, and fungi (Valdivia et al. 1996; Lorang et al., 2001; Maor et al., 2001; Lagopodi et al., 2002; Ju et al., 2005; Venard and Vaillancourt, 2007). Successful GFP expression in fungi has facilitated various investigations on fungal development, pathogenicity, and disease progression (Maor et al., 1998; Lorang et al., 2001; Lagopodi et al., 2002; Venard and Vaillancourt, 2007). Maor et al. (1998) successfully used the GFP expression in *Cochliobolus heterostrophus* to detect developmental changes that

occur in the fungus. They also followed the disease development in maize and were able to correlate the fluorescence intensity with the amount of mycelia and resulting disease severity. Venard and Vaillancourt (2007) examined the host-pathogen interaction at cellular level between maize and GFP expressing transformants of *Colletotrichum graminicola*. They described differences between the anthracnose pathogen *C. graminicola* and the non-pathogenic *Colletotrichum sublineolum* in the ability of the fungi to penetrate and colonize unwounded maize tissues. Using confocal microscopy, Sesma and Osbourn (2004) investigated pathogenicity of the rice blast fungus *Magnaporthe grisea* expressing GFP. They were able to describe the developmental steps the fungus undergo during leaf infection and the significance of root infection in disease resistance. Lagopodi et al. (2002) studied the colonization and infection of tomato roots by *Fusarium oxysporum* f. sp. *radicis-lycopersici* expressing GFP. Using the confocal laser scanning microscope, they compared the primary infection strategy of *Fusarium oxysporum* f. sp. *radicis-lycopersici* to other formae speciales of *F. oxysporum*. They demonstrated that the root tip, in contrast to the cases of other tomato wilt pathogens, is not an important site of initial infection for *F. oxysporum* f. sp. *radicis-lycopersici*.

The objective of this research was to describe infection and colonization of the hybrid bermudagrass cultivars Midlawn, and Tifway 419 (*Cynodon dactylon* x *Cyndon transvaalensis*) and an accession of African bermudagrass (*Cynodon transvaalensis*) by *O. herpotricha* expressing fluorescent proteins.

Materials and Methods

***Agrobacterium*-mediated transformation**. An *Agrobacterium tumefaciens*-mediated transformation method was used to transform the *O. herpotricha* genome (Mitchell et al., 2003; Dhulipala et al., unpublished research). The fungus was grown on potato dextrose agar (PDA) (EMD Chemicals, Gibbstown, NJ) and incubated at 25 °C for 5 days prior to transformation. Mycelia from the edge of the growing colony were fragmented using a bead-beater and incubated with *Agrobacterium tumefaciens* AGL1 carrying a binary plasmid pOHTsGFP (green fluorescent protein) or pBHt2-tdTom (red tdTomato), on which fluorescent protein genes were under the control of a strong constitutive *ToxA* promoter along with a marker gene coding for hygromycin B phosphotransferase. GFP or tdTom was inserted into *O. herpotricha* to express green or red fluorescence, respectively. Strains of *A. tumefaciens* AGL1 carrying no binary plasmid (negative control), pOHTsGFP, or pBHt2-tdTomato were grown and induced in suitable media prior to co-incubation with the hyphal fragments. To transfer the T-DNA into the fungus, the virulence-induced *A. tumefaciens* cultures were each mixed with fragmented hyphal tissues 1:1 (v:v) and co-incubated on nitrocellulose membranes overlaid on IMM (*Agrobacterium* Inducing Minimal Medium) containing appropriate antibiotics for 48 to 72 h at 20 °C. After co-incubation, the membranes were transferred and overlaid inverted mycelium-side down onto Yeast Peptone Sugar (YPS) (EMD Chemicals, Gibbstown, NJ) agar containing 50 µg/ml hygromycin B (A.G. Scientific Inc., San Diego, CA), 200 µg/ml cefotaximine (PhytoTechnology Laboratories, Shawnee Mission, KS) and 100 µg/ml timentin (PhytoTechnology Laboratories, Shawnee Mission, KS) to select fungal transformants and kill *A. tumefaciens*. Selection plates were

incubated at 20°C. Individual transformed colonies resistant to hygromycin were selected and transferred to YPS + hygromycin and incubated at room temperature for 4 to 5 days. Fungal mycelia were evaluated for fluorescence using fluorescence microscope (Nikon E800, Nikon Instruments Inc., Melville, NY). Tranformants were purified through a series of single hyphal tip isolations. DNA of transformants and wild-type *O. herpotricha* was extracted and the presence of the transgene in each transformant was confirmed using polymerase chain reaction (PCR).

Bermudagrass propagation. An aeroponics system was used to grow bermudagrass plants was used for these studies (Aeromax Jr., Foothill Hydroponics, Hollywood, CA). Plants were grown in a nutrient solution prepared following the manufacturer recommendation (Appendix Table A-1). Stolons with four to five nodes were washed several times and inserted into rockwool (Inside Sun Co., Indianapolis, IN) and grown on aeroponics system in a growth chamber (Conviron E7, Controlled Environments Ltd, Manitoba, Canada) at 25°C for three to four weeks prior to transfer to the sterile rockwools for rooting.

Inoculation of bermudagrass roots. A modified soilless assay system developed by Walker et al. (2005) was used in this study. Stolons of bermudagrass cultivars Tifway 419, Midlawn, and and a *C. transvaalensis* accession were washed several times with reverse osmosis (RO) water and rinsed with sterile distilled water twice and cut into segments containing at least a single node. Stolon segments were transferred to sterile rockwool for rooting and watered with sterile RO water. After 3 to 5 days, stolons with roots were selected and transferred individually to sterile Petri plates (100 x 15 mm,VWR, Batavia, IL) lined with double layer of wet white paper towel (enMotion High

Capacity Roll, enMotion, Syracuse, NY) and placed on a microscope slide (3 x 1 x 1mm, Fisher Scientific Inc., Pittsburgh, PA) (Appendix 1). The plates were saturated with sterile RO water. A single root from each stolon was inoculated with mycelial agar plug (approximately 0.25 mm in diameter) of GFP *O. herpotricha* grown on hygromycin-containing PDA. Agar plugs were placed near the root surface. One non-inoculated plant for every five inoculated replicates was used as a control. Plates were sealed with parafilm (Pechiney Plastic Packaging, Menasha, WI) and were incubated upright in a growth chamber (Conviron E7) at 17 °C. This study was repeated four times.

Inoculation of bermudagrass roots for disease severity evaluation. Mycelia of *O. herpotricha* expressing tdTom (red) fluorescent protein were scraped from 3 PDA plates. Mycelia were transferred into 50 ml sterile water with Danitol (100 µL/L) and fragmented using a blender (Waring Commercial, Torrington, Connecticut) for 5 min. The mycelial fragment suspension was transferred into sterile 250 ml Pyrex beaker (Corning Inc., Lowell, MA). Stolons were grown for rooting on rockwool as described previously. Plants of each cultivar were washed with sterile RO water twice and 10 plants were tied per cultivar with sterile cotton string. Bundles were washed once with sterile RO water and then dipped into the mycelial fragment suspension for 2 min. Bundles were transferred and spread onto a sterile double layer of wet paper towels. Plants were aligned side by side, and the wet paper towel was rolled and inserted into a sterile 50 ml polypropylene centrifuge tube (Fisher Scientific, Pittsburgh, PA). All plants were incubated in the growth chamber (Conviron E7) for 14 days at 17 °C and 12 h light. This study was repeated once.

Microscopy. Roots were observed microscopically numerous times from 1 day post-inoculation (DPI) to 14 DPI. Some roots were embedded in 3% agarose (Promega Corp., Madison WI) and hand-sectioned with a surgical blade (Bard-Parker, Becton Dickinson Acute Care, Franklin Lake, NJ). Inoculated root surfaces, transverse sections, and longitudinal sections were mounted in water and observed using a Nikon Eclipse E-800 epi-fluorescence microscope equipped with mercury-vapor lamp as light source and filter sets including G-2E/C TRITC (EX 528-553 nm/EM 600-660 nm) for red (tdTom) fluorescent protein and root autofluorescence detection, UV-2E/C DAPI (EX 330-380 nm/EM 435-485 nm) for UV autofluorescence and necrosis detection, Endow GFP HYQ longpass (EX 450-490 nm/EM >500 nm) for GFP and cell autofluorescence detection and GFP-3035B (EX 457-487 nm/EM 503-538 nm) for GFP detection. Autofluorescence of cells was used to visualize the healthy root cells. Necrotic responses were detected as non-fluorescent dead cells.

Digital images were captured with using a monochrome QImaging Retiga 2000R charge-coupled device (CCD) camera (Quantitative Imaging Corp., Surrey, BC, Canada). Location of infection and colonization at the cellular level were observed. Differences or similarities in infection and colonization of the three different cultivars were determined. Composites images were constructed using QCapture Pro version 5.1.1.14 (Quantitative Imaging Corp., Surrey, BC, Canada) software to combine and pseudocolor different layers of images captured with different filters. Macroscopic images of roots were captured using a Nikon SMZ-2T stereomicroscope (Nikon Inc., Tokyo, Japan) with a Olympus DP71 CCD camera (Olympus Imaging America Inc., Center Valley, PA)

Disease severity ratings. Bermudagrasses were assessed for necrotic response to *O. herpotricha* at 14 DPI. Macroscopic lesions were rated on a 0 to 5 scale where: 0 = no root discoloration, 1 = yellow root discoloration without necrotic lesions, 2 = scattered small light tan brown lesions (<25% of root), 3 = scattered solid or limited dark lesions (<25% of root), 4 = large dark solid lesions and very visible (<50% of root), and 5 = necrosis on almost entire root (>50% of root). Microscopic lesions were observed using epi-fluorescence at 100x magnification with the UV filter that detects necrosis as black and healthy root tissue blue. A scale of 0 to 6 was used to assess necrosis where: 0 = no symptoms, 2 = less severe with only small necrotic lesions covering less than 25% of the root, 4 = moderately severe with necrotic lesions covering less than 50% of the root, and 6 = extremely severe with necrotic lesions covering more than 50% of the root. Root colonization was assessed based on composite images of monochrome images captured with UV, GFP and TRITC filters. A scale of 0 to 6 was used where: 0 for no infection or colonization, 2 = colonization with few ectotrophic hyphae on root, 4 = less aggregated and loosely interwoven hyphae covering less than 50% of the root surface, and 6 = densely interwoven and aggregated hyphae covering more than 50% of the root surface. Transverse sections of the roots were examined using a Nikon Eclipse E-800 epi-fluorescence microscope as described previously. Statistical analysis was conducted with SAS (version 9.1, SAS Institute, Cary, NC) to evaluate cultivar effects for the different methods over experiments. Treatment means within a method were compared using the Fisher's protected least significant difference test.

Results

Transformation of *Ophiosphaerella herpotricha*. Six isolates of *O. herpotricha* with GFP and 13 isolates of tdTom were generated. Epifluorescence microscopy revealed bright fluorescence from the isolates with either GFP or tdTom. Hyphae were sterile, simple, and melanized. On agar plates, mycelia of GFP expressing isolates appeared slightly yellow, tdTom expressing isolates were light pink with ambient lighting, and wild-type isolates were white to yellow. Mycelia of all isolates turned to tan brown after 7 days.

Establishment of Infection and Colonization. The initial processes of hyphal penetration and infection were similar on all three cultivars. At 1 to 2 days post-inoculation, hyphae from the agar plug started to grow but did not appear to orient towards the roots (Figs. 1A, 9A, 16A). Hyphae that grew came into contact with the root surface without pre-attachment or recognition of root hairs 1 to 2 days post-inoculation(Figs. 1B, 9B, 16B). Some hyphae attached to the surface of roots and directly penetrated the epidermis while some became ectotrophic hyphae and moved intercellularly along the groove in between the epidermal cells and transverse junctions of epidermal cells prior to penetration. Host cell responses occurred after penetration of epidermal cells and colonization. For all the cultivars, penetrated epidermal cells remain alive initially (Figs. 1C, 9C, 16C). Formation of hyphopodia similar to those of *Gaeummanomyces graminis* or infection cushions as previously described (Asher et al., 1981; Smiley, et al., 2005; Clarke and Gould, 1993) were not observed prior to penetration and infection. However, pointed hyphal ends similar to those described as

infection pegs for *Magnaporthe* and *Gaeummanomyces graminis* were observed as the fungus penetrated the epidermis (Figs. 1B, 9B).

Tifway 419 roots. At 2 DPI, the fungus started to directly colonize the root epidermis intercellularly (Fig. 2, A and B). Aggregates of hyphae were formed after initial penetration that appeared as a mat or stroma embedded in and around epidermal cells (Fig. 2B). Ectotrophic hyphae emerged from these aggregates and colonized adjacent epidermal cells. At 2 DPI, the infection was generally restricted to the epidermal layer (Fig. 2, A and B) and the ectotrophic hyphae started to form branches and loosely interwoven networks of hyphae at 3DPI (Fig. 1C). After the initial infection, rapid fungal growth occurred at 4 DPI (Fig. 1D). Before 4 DPI, the majority of the roots observed appeared healthy with few macroscopic necrotic symptoms. However, after 4 DPI, extensive necrotic microlesions appeared on colonized roots.

Macroscopic root lesions generally started to appear and develop into larger lesions at 5 to 8 DPI. At 5 DPI, the root epidermis appeared necrotic near hyphal infection (Fig. 3). Dark brown root lesions 4 to 8 mm in length were observed at 7 DPI. At 8 DPI, a densely interwoven network of hyphae was observed with severe necroses at the site of initial infection (Fig. 4). Mats of aggregated hyphae appeared on roots at 8 DPI. Underlying cortical cells of Tifway 419 root appeared to shrink, an early indication of cell collapse near hyphal infection at 8 DPI (Fig. 5). The root epidermis collapsed around the hyphae was observed (Fig. 5). Infections of lateral roots of Tifway 419 were initiated upon emergence and hyphae tended to clump and cause necrosis in epidermal region.

For lesions with dark discoloration, the associated hyphal network was dense and extensive. Hyphal aggregates were observed near the initial infection site and in necrotic lesions 10 DPI (Fig. 6). Colonization was mostly intercellular among epidermal and cortical layers but some intracellular growth was also observed. Colonization of the inner cortical layer occurred by 10 DPI (Fig. 7). Lesions on Tifway roots were darker and started to collapse. Longitudinal sections revealed that transverse cortical colonization is limited after initial penetration (Fig. 8). Hyphal growth in the cortex occurred mainly longitudinally in multihyphal strands or as single hyphae colonizing both intra- and intercellularly. The necrotic responses did not limit fungal growth both intra- or intercellularly. There was less aggregation of ectotrophic hyphae at the point of emergence of secondary or lateral roots however necrosis was present in these locations. Transverse sections of Tifway roots revealed hyphae in the cortical layer but none in the vascular region, which remained free of infection (Fig. 7). Beyond the margin of lesions, non-necrotic intercellular infections were observed.

Midlawn roots. After growing out of the agar plug 2 DPI, hyphae came in contact with the root epidermis (Fig. 9, A and B). At 2 DPI, the infection was generally restricted at epidermal layer (Figs. 9B, 10A, 10B). Ectotrophic hyphae appeared to grow along the root surface longitudinally at 3DPI (Fig. 9C). After initial infection, rapid fungal growth occurred at 4 DPI (Fig. 9D). Aggregates of hyphae appeared embedded in and around epidermal cells (Fig. 9D). Ectotrophic hyphae emerged from these aggregates and colonized adjacent epidermal cells. Microscopic lesions appeared on colonized parts of the root at 4 DPI. Macroscopic root lesions generally appeared and developed into larger lesions between 5 to 8 DPI. At 5 DPI, transverse sections of Midlawn roots

revealed hyphae in the cortex but infected cells adjacent to intercellular hyphae did not appear necrotic (Fig. 11). Dark brown lesions, approximately 3 mm in length were observed at 7 DPI. Ectotrophic hyphae and loose aggregates of hyphae were observed in the severe necrosis at the site of initial infection at 8 DPI (Fig. 12). Cell wall collapse was observed in the epidermal and cortical layers in the presence of extensive colonization at 8 DPI (Fig. 13). Overall, Midlawn showed a slower reaction to infection by *O. herpotricha* and appeared more tolerant than Tifway 419.

The hyphal network was less aggregated in Midlawn than in Tifway 419 but necrotic lesions were dark and similar to Tifway (Fig. 14). Lesions along Midlawn roots were dark and started to collapse (Fig. 14). Colonization of the inner cortical layer occurred but generally not until 10 DPI (Fig. 15). Transverse sections of roots contained hyphal growth in between cortex cells and infected cells adjacent to intercellular hyphae appeared necrotic (Fig. 15). At 10 DPI, additional large dark necrotic areas were observed in non-colonized areas of the roots and green fluorescence from hyphae of older infections appeared less intense. The necrotic responses did not limit fungal growth either intra- or intercellularly. The vascular region of Midlawn remained free of infection at 10 DPI (Fig. 15). Beyond the margin of lesions, non-necrotic infections were observed.

***Cynodon transvaalensis* roots**. At 2 DPI, the fungus came in contact with the root epidermis (Fig. 16, A and B). Ectotrophic hyphae appeared to grow along root surfaces intercellularly near the inoculation point. At 2 DPI, the infection was generally restricted to the epidermal layer (Fig. 17). Rapid fungal growth occurred at 3 DPI. Aggregates of hyphae appeared along the root surface and formed a network of hyphae (Fig. 16C). At 4 DPI, a loose network of ectotrophic hyphae was observed on root

surfaces (Fig. 16D). Close observation of the primary infection site revealed microscopic necrotic lesions. The microscopic lesions observed appeared only at the point of initial penetration. At 5 DPI, the cortical layer near and around the vascular core of *C. transvaalenis* was intensively colonized (Fig. 18A). Tan brown macroscopic lesions on root surfaces were observed at 8 DPI. Close observation of these lesions revealed loose aggregates of hyphae on the root surfaces (Fig. 19). Vascular infection of *C. transvaalensis* roots was observed at 8 DPI within the endodermis near the xylem-phloem bundle (Fig. 20A); however the vascular region did not appear necrotic (Fig. 20B). Cell wall collapse was not observed in infected *C. transvaalensis* roots, either in the epidermis, cortex, or vascular core, even with extensive colonization.

A network of hyphae on *C. transvaalensis* roots was observed 10 DPI, but hyphae were less aggregated than those on Tifway 419 and Midlawn roots (Fig. 21). Fluorescence signal from hyphae on roots became weaker as the root tissues turned yellow. Macroscopic lesions on the root were observed near the initial infection site and were tan to brown in color. At 14 DPI, the fungus extensively colonized the epidermis, cortex, and stele (Fig. 22) but infected cells were not necrotic. The fungus appeared to colonize the parenchyma cells of the pith region (Fig. 22).

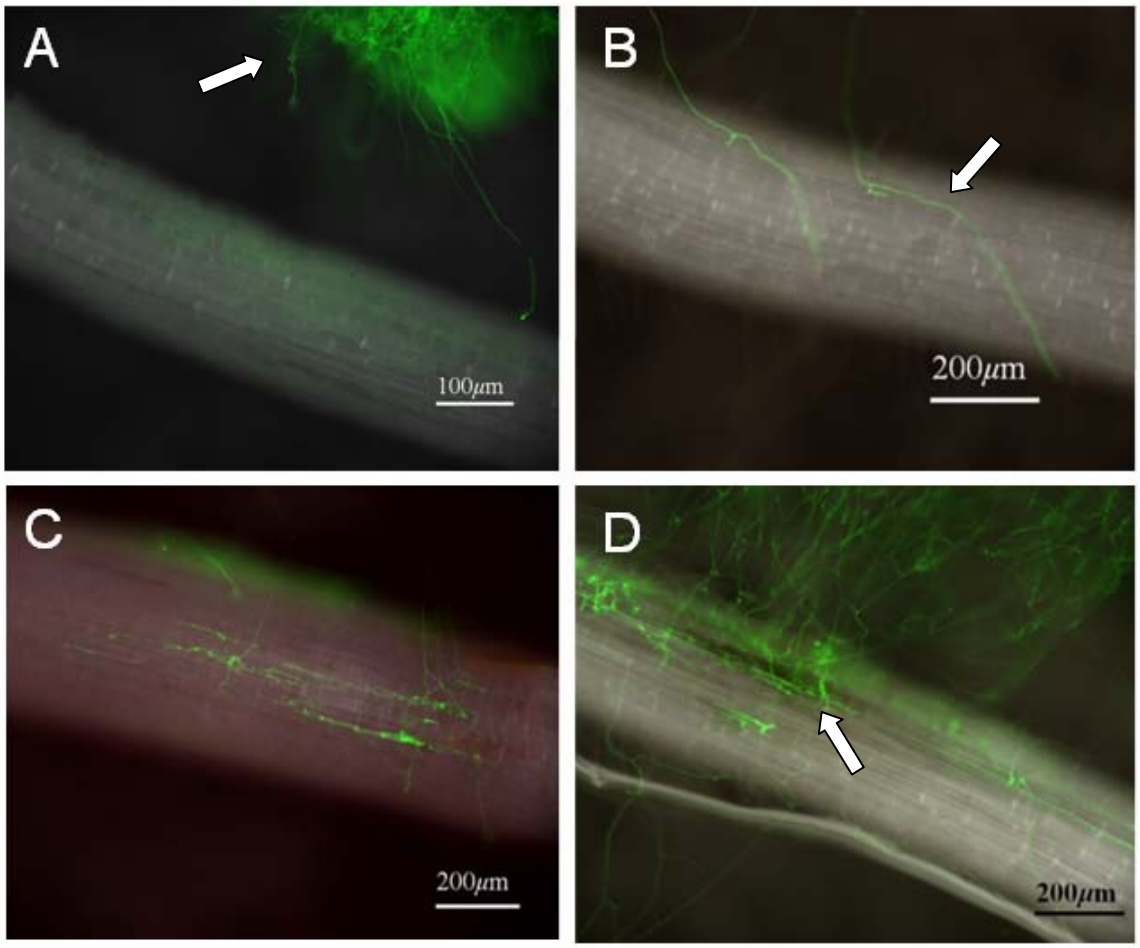

Figure 1. Establishment of infection and colonization of Tifway 419 by an isolate of *Ophiosphaerella herpotricha* expressing green fluorescent protein. A) Tifway 419 root and agar plug with mycelium (arrow) at 1 day post-inoculation (DPI); B) ectotrophic hyphae (arrow) on the root surface at 2 DPI; C) primary infection and early colonization at 3 DPI; and D) colonized root surface with necrosis (arrow) at 4 DPI.

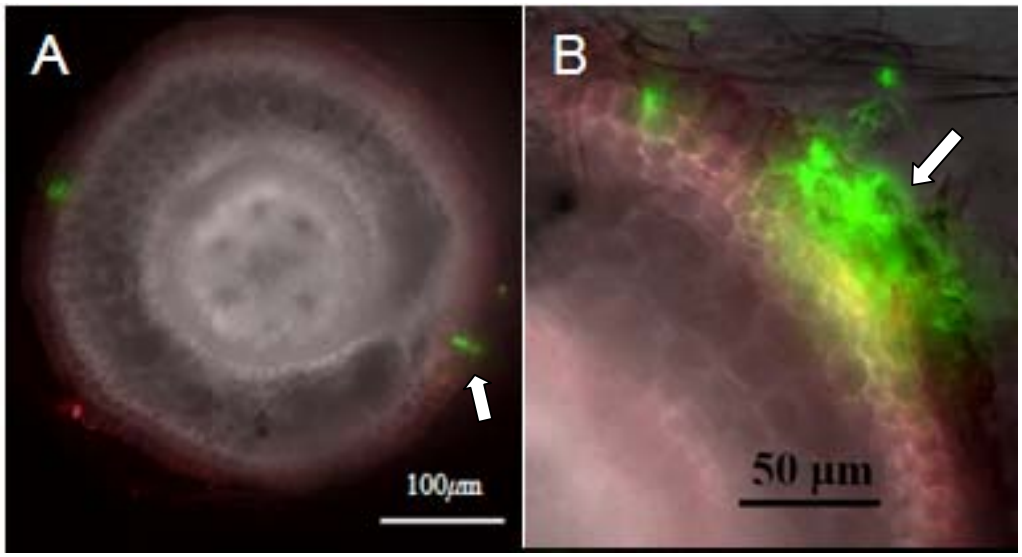

Figure 2. Transverse root sections of Tifway 419 inoculated with an isolate of *Ophiosphaerella herpotricha* 2 days post-inoculation. A) Tifway 419 with ectotrophic hyphae (arrow) at the epidermal layer; B) Tifway 419 with hyphal aggregates (arrow) in the epidermal layer.

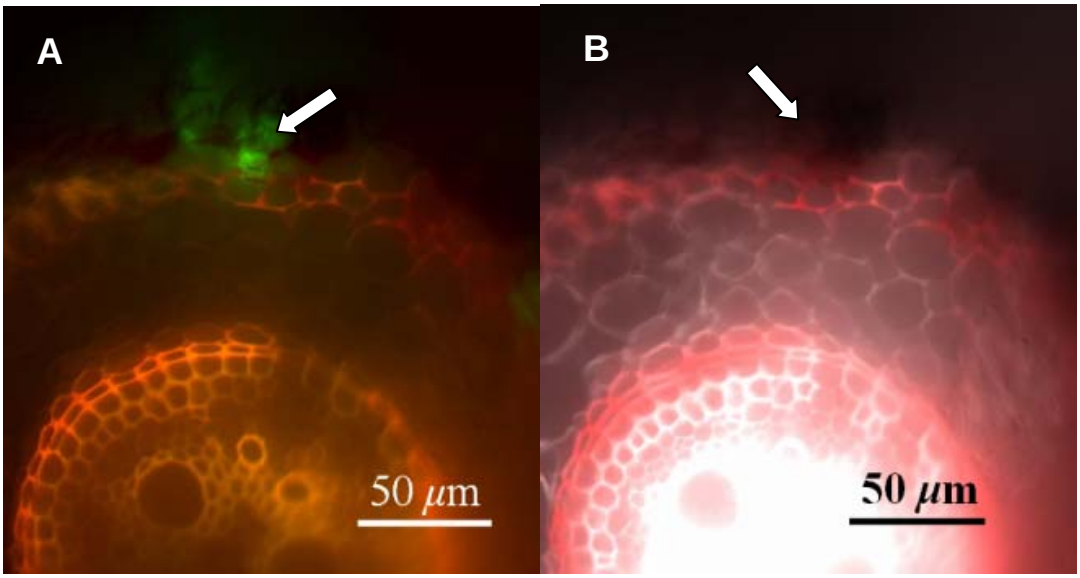

Figure 3. Transverse root sections of Tifway 419 inoculated with an isolate of *Ophiosphaerella herpotricha* expressing green fluorescent protein at 5 days post-inoculation. A) hyphae (arrow) in the root epidermis (GFP and TRITC composite); and B) colonized cells exhibiting necrosis (arrow) (UV and TRITC composite).

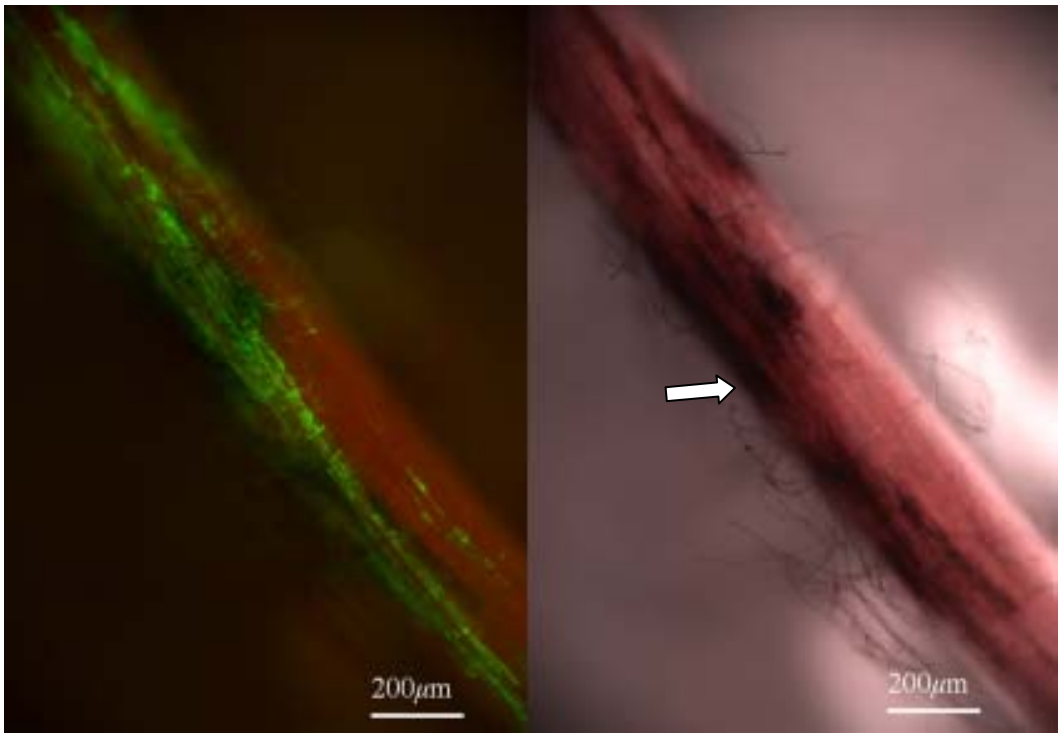

Figure 4. Infection and colonization of Tifway 419 by an isolate of *Ophiosphaerella herpotricha* expressing green fluorescent protein at 8 days post-inoculation. Left – root surface colonized by the fungus (GFP and TRITC composites); and Right - root surface colonized by the fungus exhibiting necrotic lesions (arrow) (UV and TRITC composites).

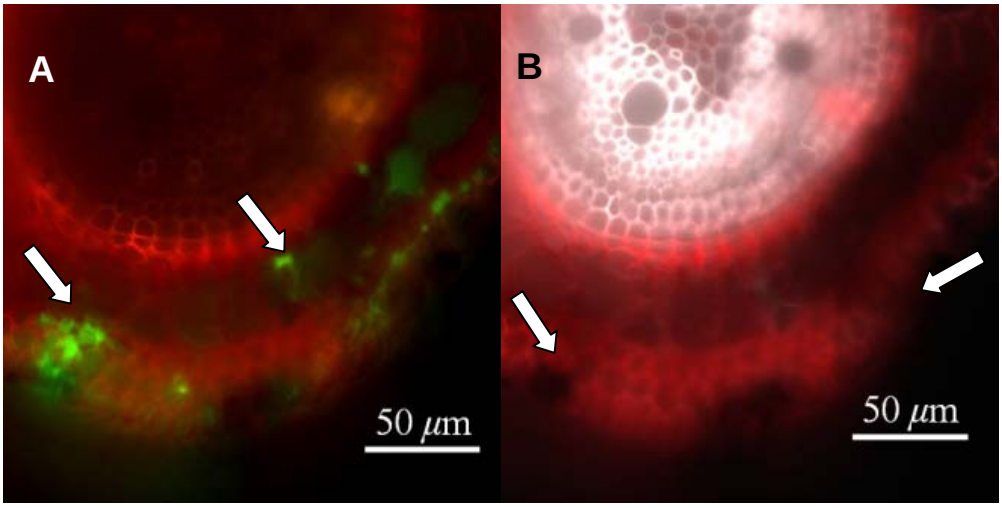

Figure 5. Transverse root sections of Tifway 419 inoculated with an isolate of *Ophiosphaerella herpotricha* expressing green fluorescent protein at 8 days post-inoculation. A) hyphal growth (arrows) in the root epidermis and cortex (GFP and TRITC composite); and B) colonized root exhibiting cell collapse and necrosis (arrows) (UV and TRITC composites).

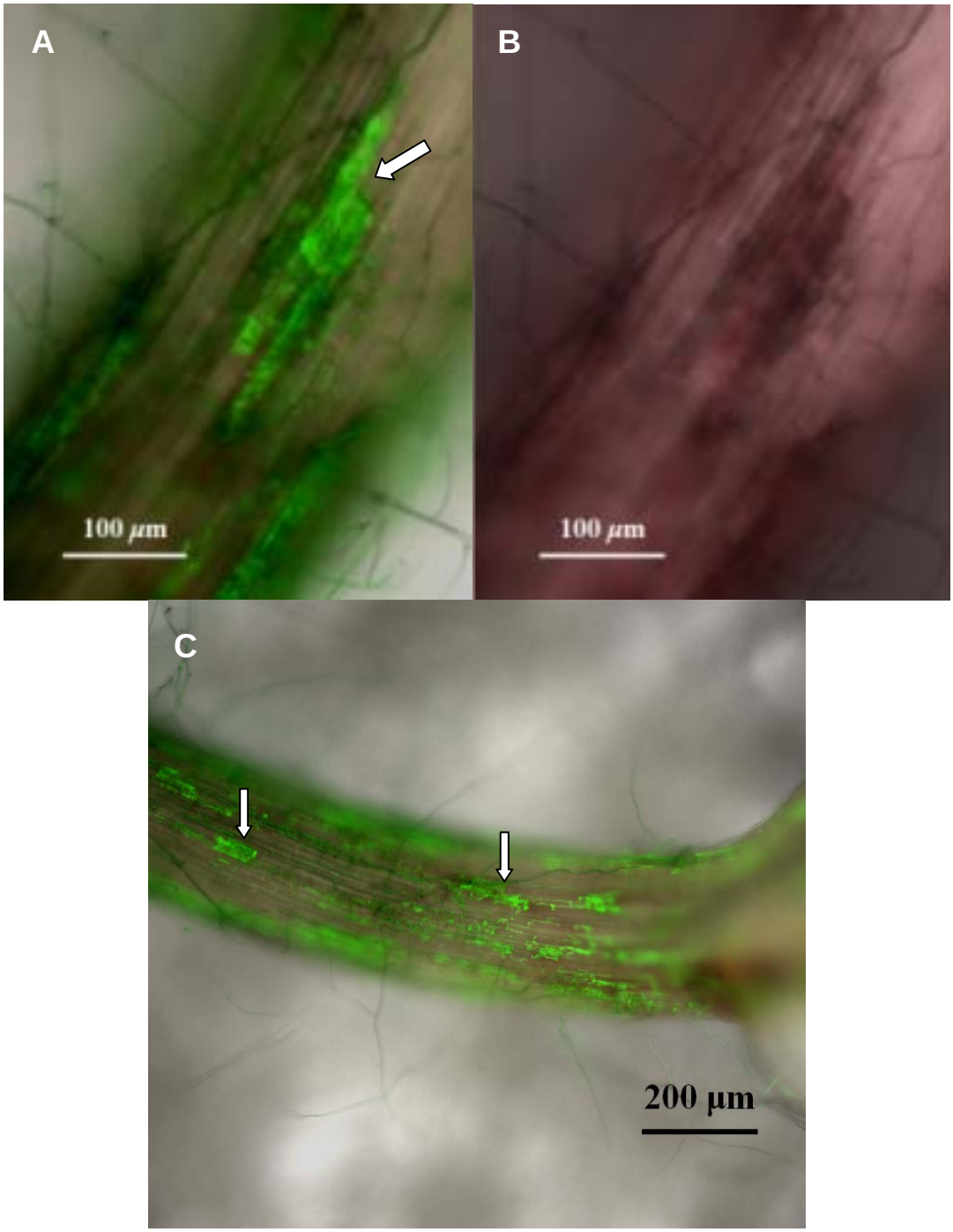

Figure 6. Root colonization and necrotic lesion formation on Tifway 419 at 10 days post-inoculation. A) root surface colonized by *Ophiosphaerella herpotricha* with hyphal aggregates (arrow); B) root surface with necrosis caused by *O. herpotricha*; and C) mat-like aggregates of hyphae (arrows) on root surface.

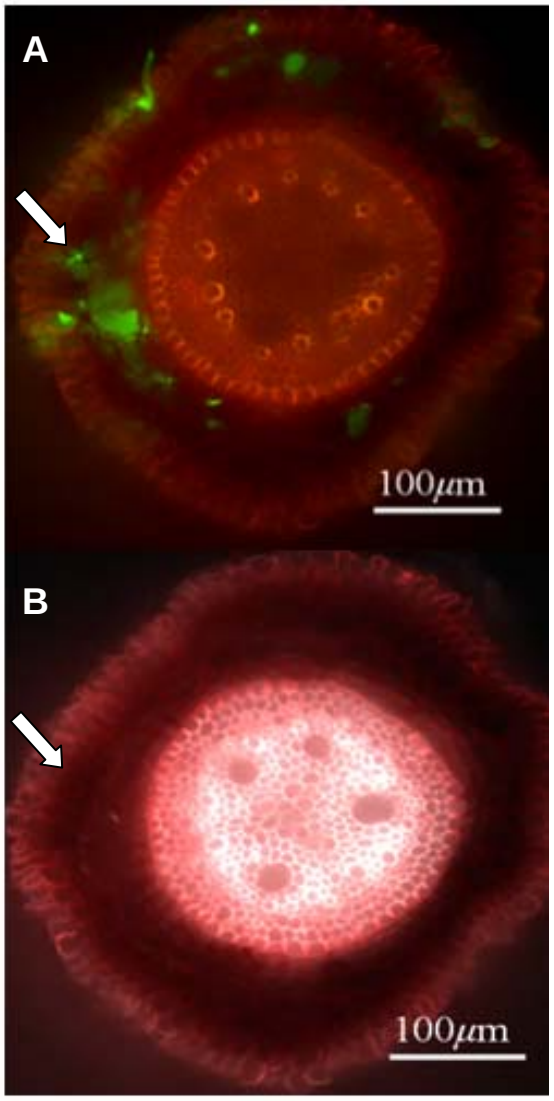

Figure 7. Transverse root sections showing colonization and necrotic lesion formation in Tifway 419 at 10 days post-inoculation. A) *Ophiosphaerella herpotricha* hyphae (arrow) in the root cortex (GFP and TRITC composite); and B) colonized root with necrotic cortex (arrow) (UV and TRITC composite).

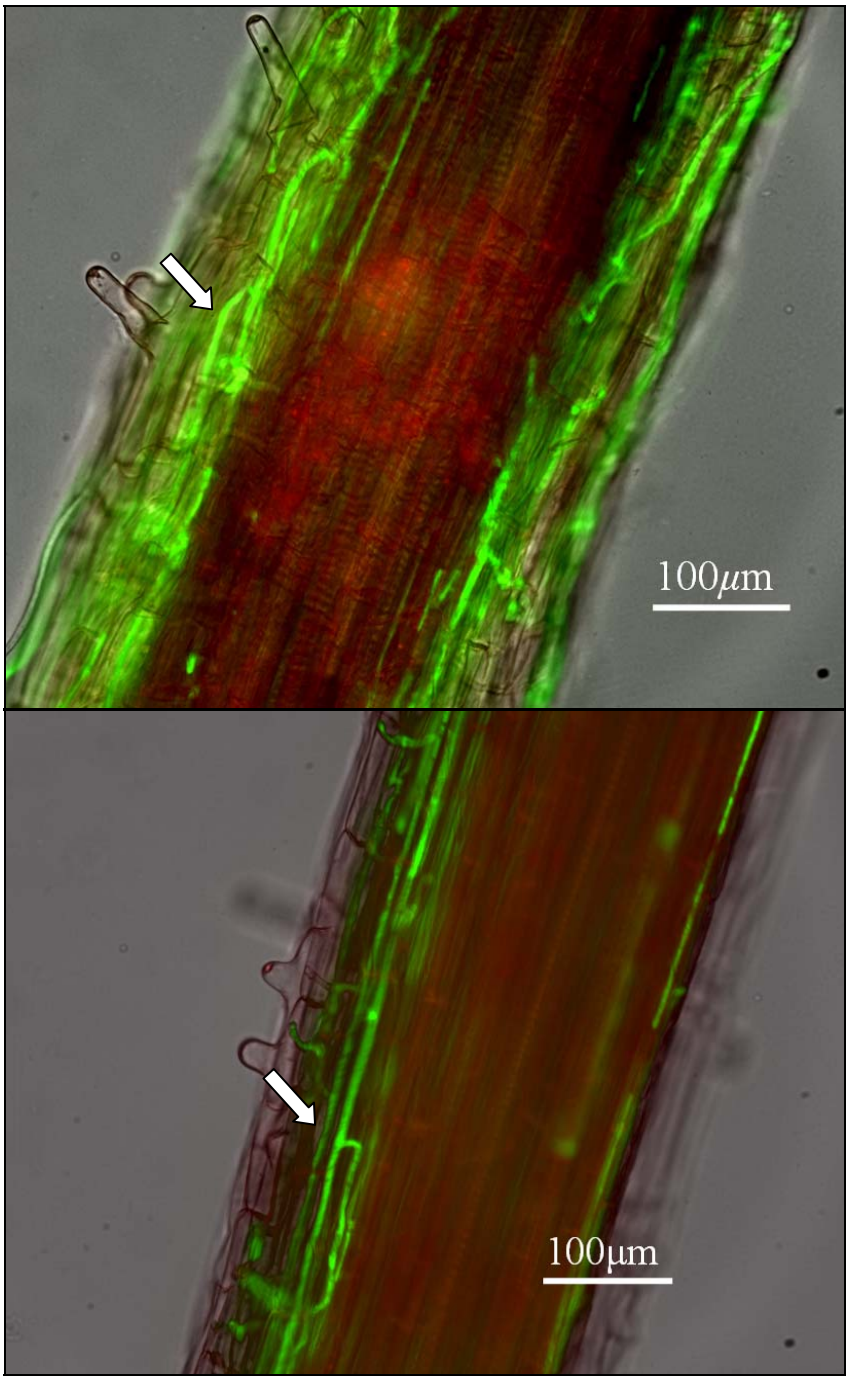

Figure 8. Longitudinal root sections showing colonization in Tifway 419 at 10 days post-inoculation. Longitudinal growth of multihyphal strands or single hyphae of *Ophiosphaerella herpotricha* (arrows) in the root cortex.

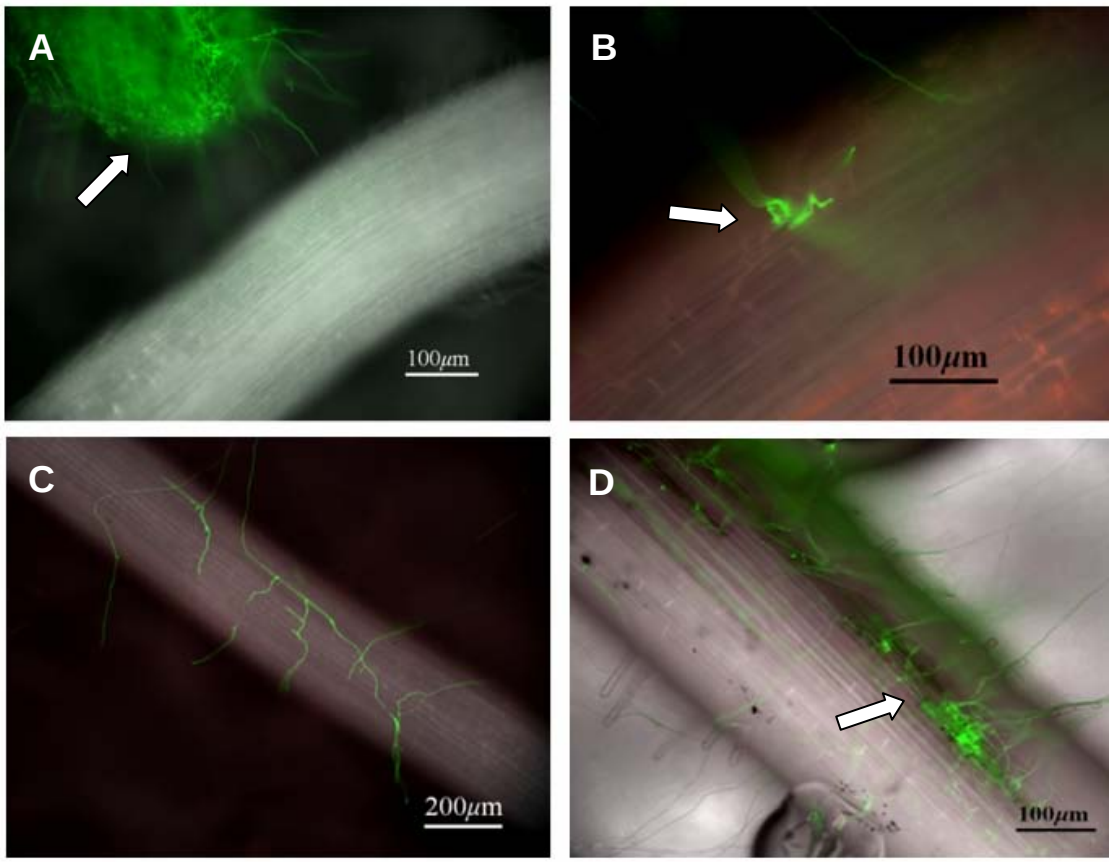

Figure 9. Establishment of infection and colonization of Midlawn by an isolate of *Ophiosphaerella herpotricha* expressing green fluorescent protein. A) Midlawn root and agar plug with mycelium (arrow) at 1 day post-inoculation (DPI); B) ectotrophic hyphae (arrow) on the root surface at 2 DPI; C) primary infection and early colonization at 3 DPI; and D) colonized root surface with necrosis (arrow) at 4 DPI.

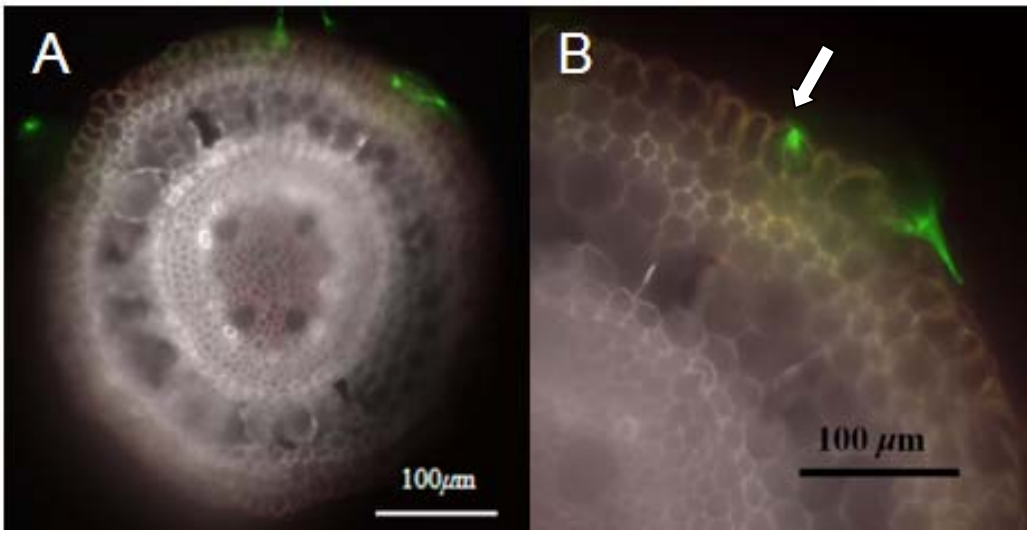

Figure 10. Transverse root sections of Midlawn inoculated with an isolate of *Ophiosphaerella herpotricha* expressing green fluorescent protein 2 days post-inoculation. A) Midlawn with ectotrophic hypahe on root surface; and B) Midlawn with ectotrophic hyphae (arrow) at the epidermal layer.

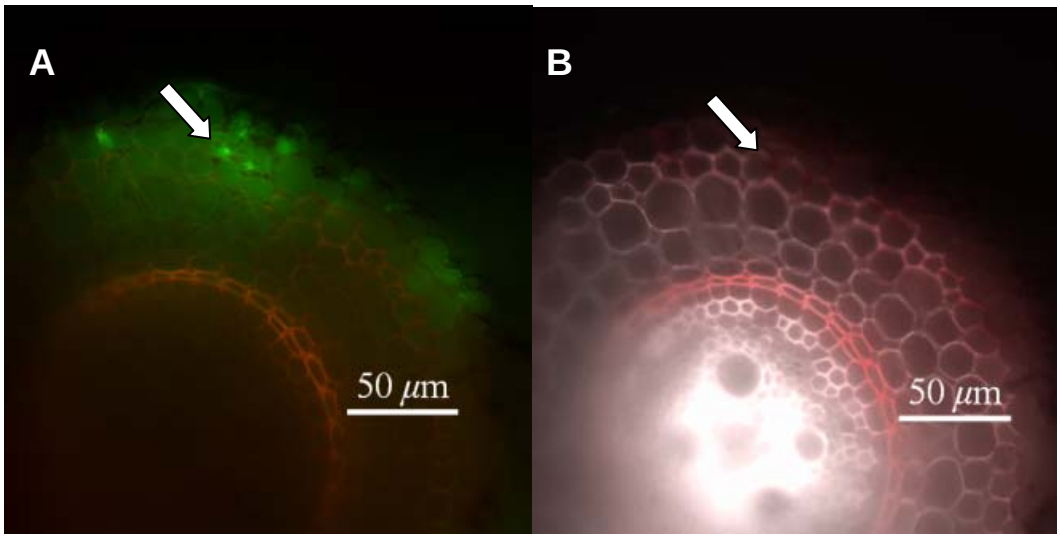

Figure 11. Transverse root sections of Midlawn inoculated with an isolate of *Ophiosphaerella herpotricha* expressing green fluorescent protein at 5 days post-inoculation. A) hyphae (arrow) at the root cortex (GFP and TRITC composite); and B) colonized cells exhibiting necrosis (arrow) (UV and TRITC composite).

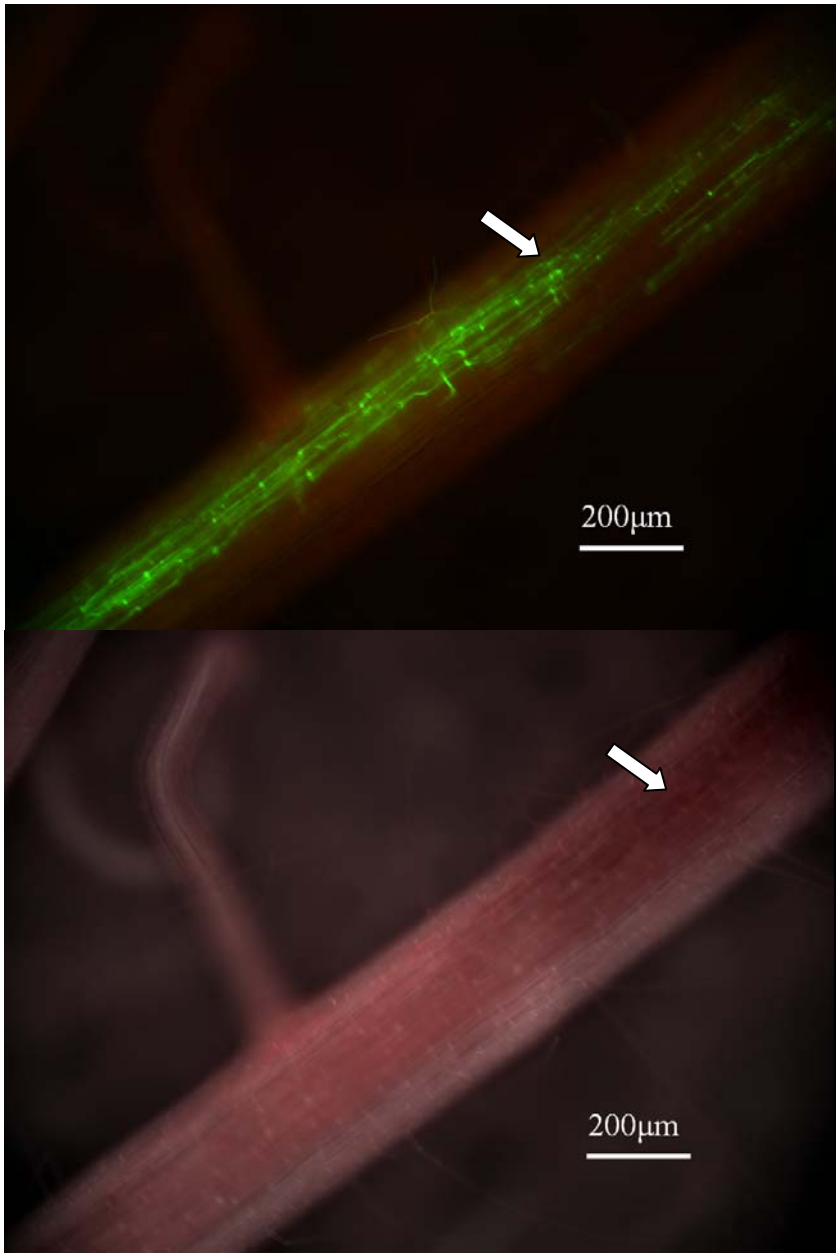

Figure 12. Infection and colonization of Midlawn by an isolate of *Ophiosphaerella herpotricha* expressing green fluorescent protein at 8 days post-inoculation. Left – root surface colonized by the fungus (arrow) (GFP and TRITC composite); and Right – root surface colonized by the fungus exhibiting necrotic lesion (arrow) (UV and TRITC composite).

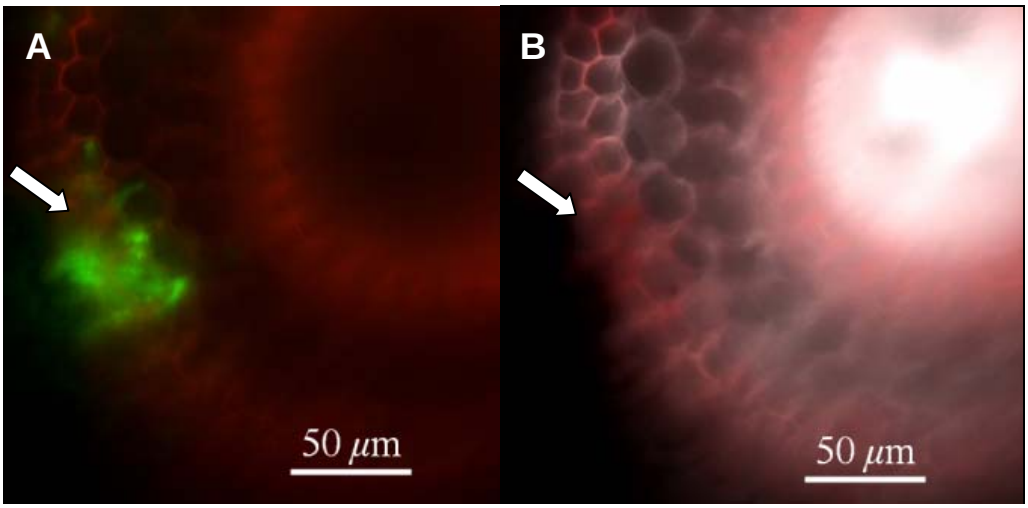

Figure 13. Transverse root sections of Midlawn inoculated with an isolate of *Ophiosphaerella herpotricha* expressing green fluorescent protein at 8 days post-inoculation. A) hyphal growth (arrow) in the root epidermis and cortex (GFP and TRITC composite); and B) necrotic epidermal and cortical cells (arrow) (UV and TRITC composite).

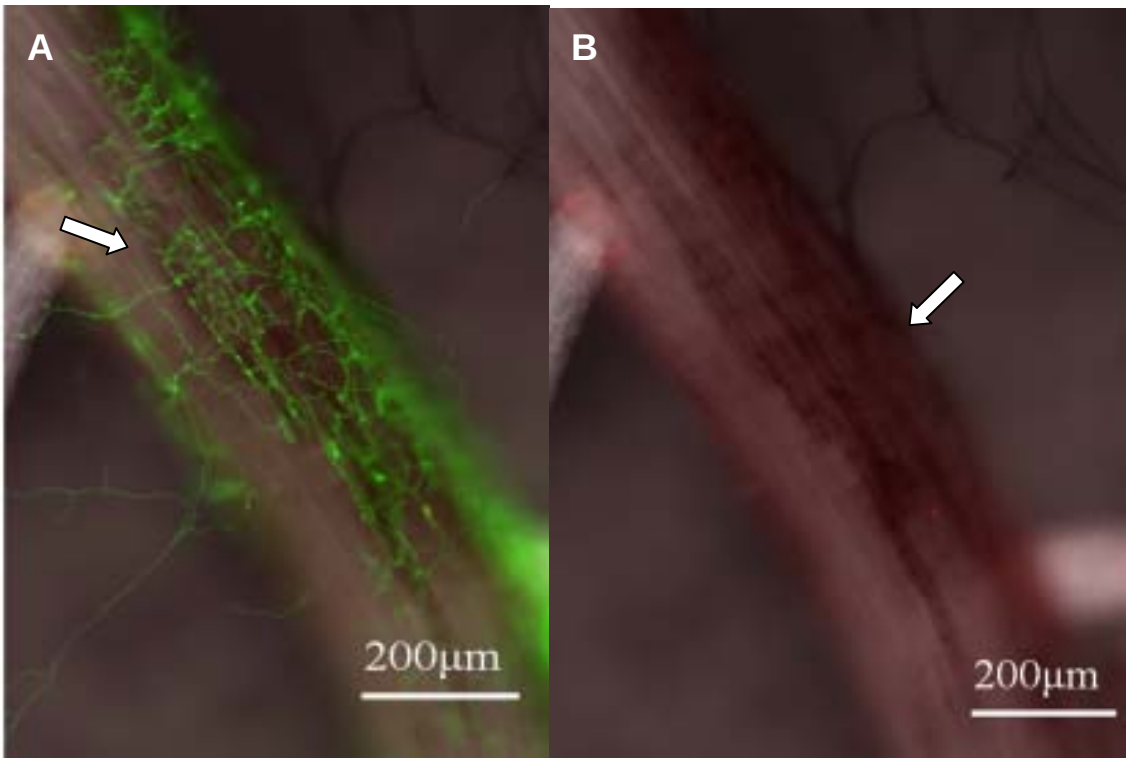

Figure 14. Root colonization and necrotic lesion formation on Midlawn at 10 days post-inoculation. A) root surface (arrow) colonized by *Ophiosphaerella herpotricha* with network of hyphae and hyphal aggregates; and B) root surface with necrosis (arrow) caused by *O. herpotricha*.

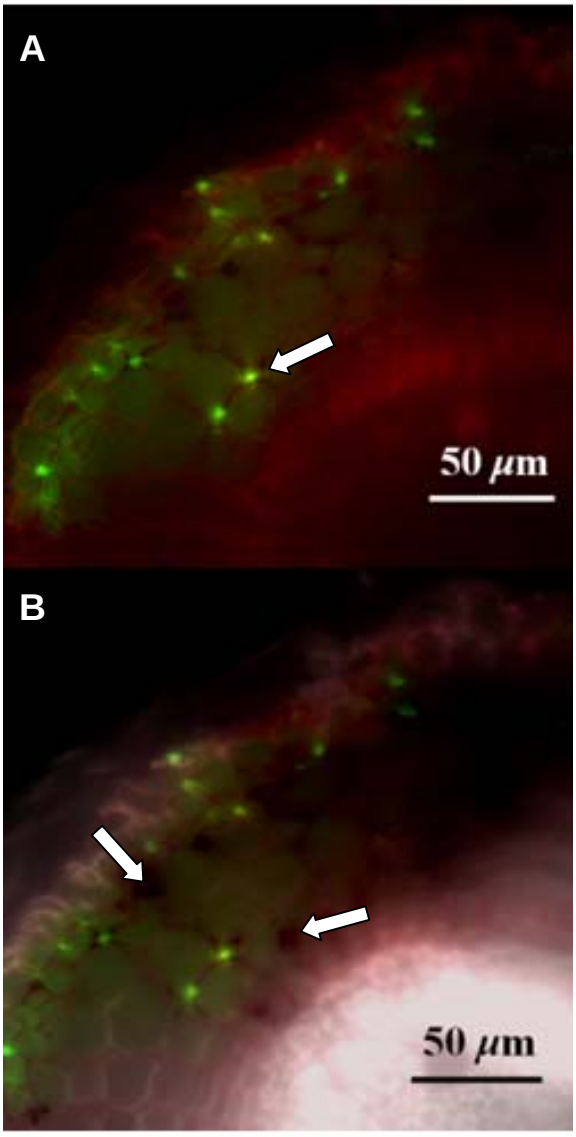

Figure 15. Transverse root sections showing colonization and necrotic lesion formation in Midlawn at 10 days post-inoculation. A) intercellular hyphal growth (arrow) of *Ophiosphaerella herpotricha* in the root cortex (GFP and TRITC composite); and B) necrotic cortical cells (arrows) of the infected root (GFP, UV and TRITC composite).

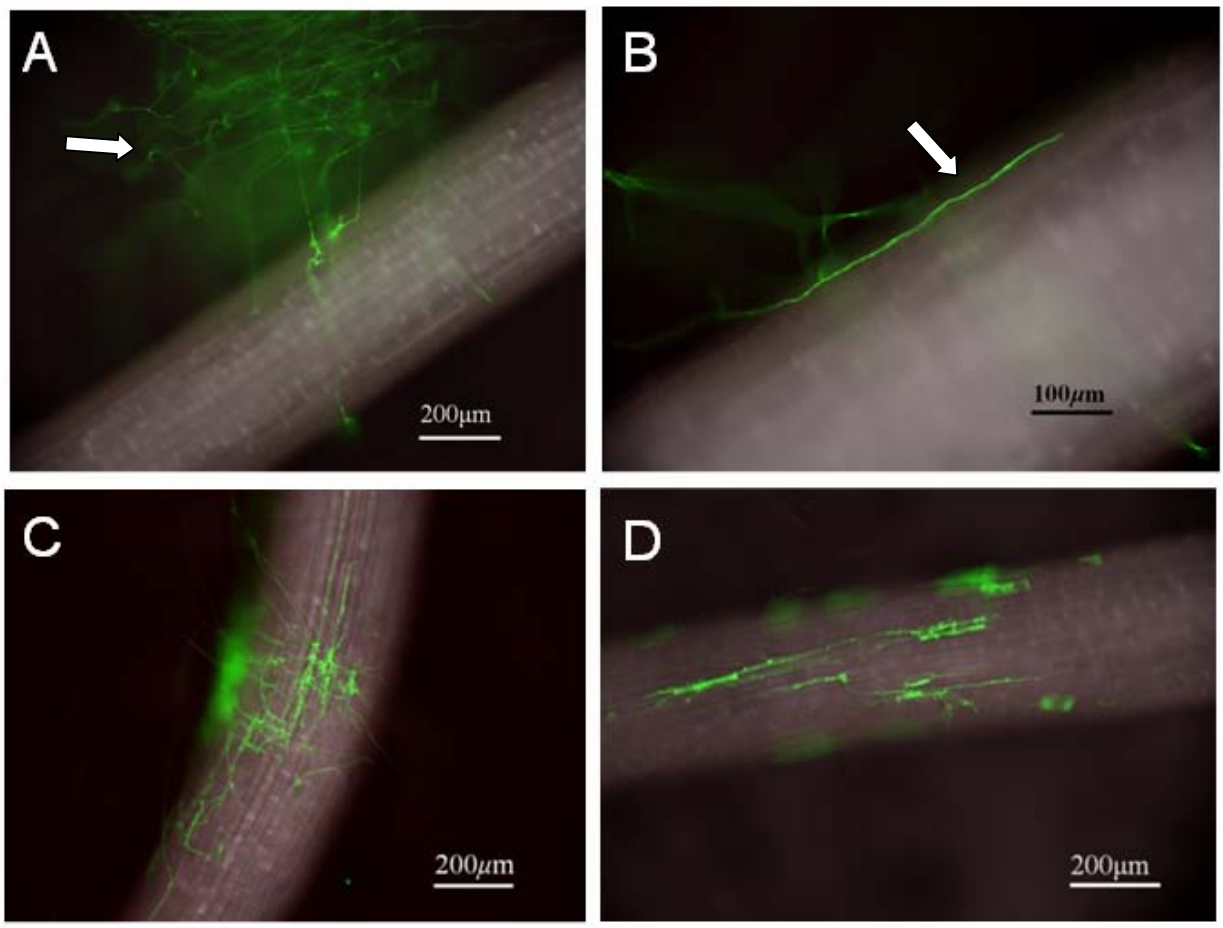

Figure 16. Establishment of infection and colonization of *Cynodon transvaalensis* by an isolate of *Ophiosphaerella herpotricha* expressing green fluorescent protein. A) *Cynodon transvaalensis* root and agar plug with mycelium (arrow) at 2 days post-inoculation (DPI); B) ectotrophic hyphae (arrow) on the root surface at 2 DPI; C) primary infection and early colonization at 3 DPI; and D) colonized root surface with no necrosis at 4 DPI.

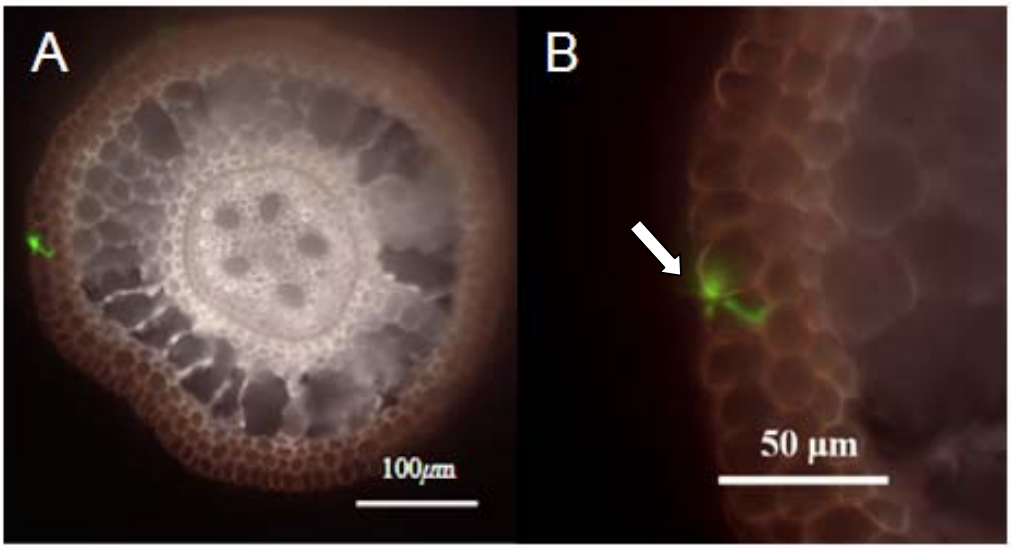

Figure 17. Transverse root sections of *Cynodon transvaalensis* inoculated with an isolate of *Ophiosphaerella herpotricha* expressing green fluorescent protein 2 days post-inoculation. A) *C. transvaalensis* with hyphae in the epidermal layer; and B) hyphal penetration (arrow) of *C. transvaalensis* epidermal layer.

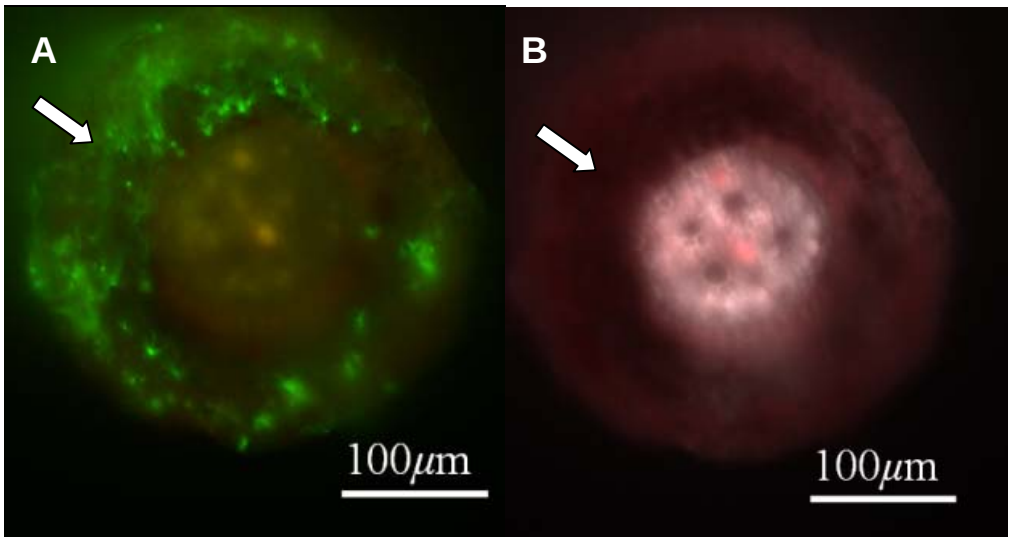

Figure 18. **Transverse root sections of *Cynodon transvaalensis* inoculated with an isolate of *Ophiosphaerella herpotricha* expressing green fluorescent protein at 5 days post-inoculation**. A) hyphal growth (arrow) in the root cortex (GFP and TRITC composite); and B) colonized root cortex exhibiting necrosis (arrow) (UV and TRITC composite).

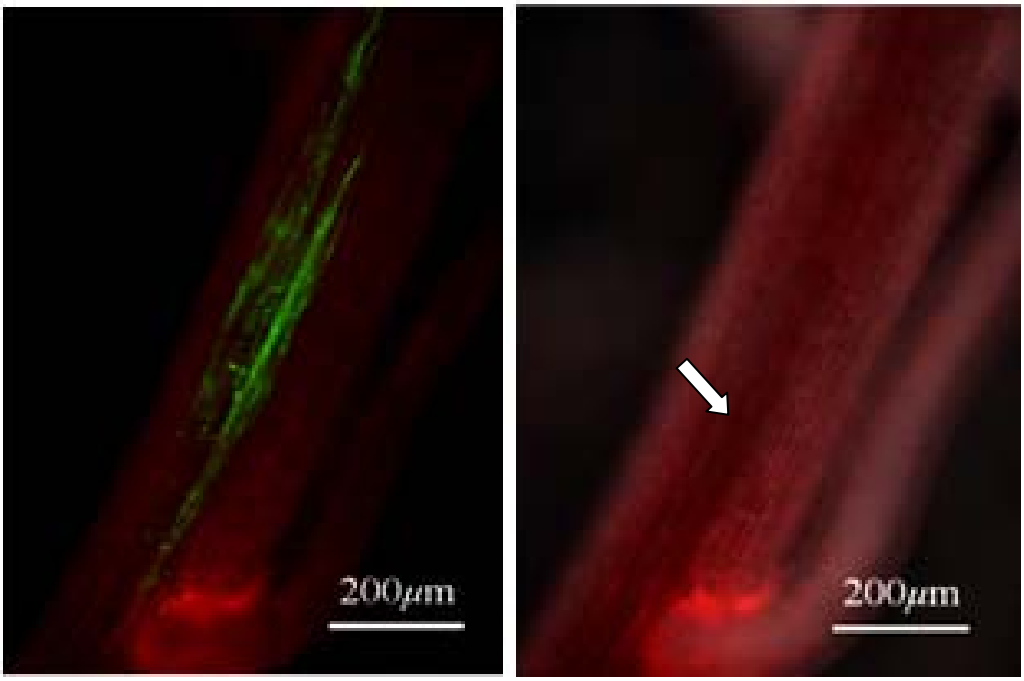

Figure 19. Infection and colonization of *Cynodon transvaalensis* by an isolate of *Ophiosphaerella herpotricha* expressing green fluorescent protein at 8 days post-inoculation. Left – root surface colonized by the fungus (GFP and TRITC composite); and Right – root surface colonized by the fungus exhibiting necrotic lesion (arrow) (UV and TRITC composite).

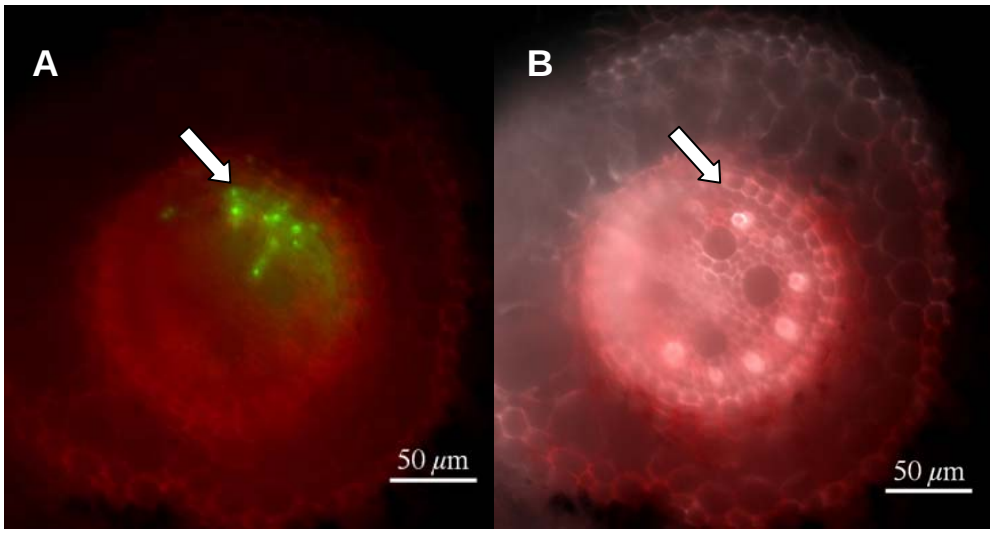

Figure 20. **Transverse root sections of *Cynodon transvaalensis* inoculated with an isolate of *Ophiosphaerella herpotricha* expressing green fluorescent protein at 8 days post-inoculation**. A) intercellular hyphae (arrow) in the vascular region of infected root (GFP and TRITC composite); and B) colonized vascular region of the root with no necrosis (UV and TRITC composite).

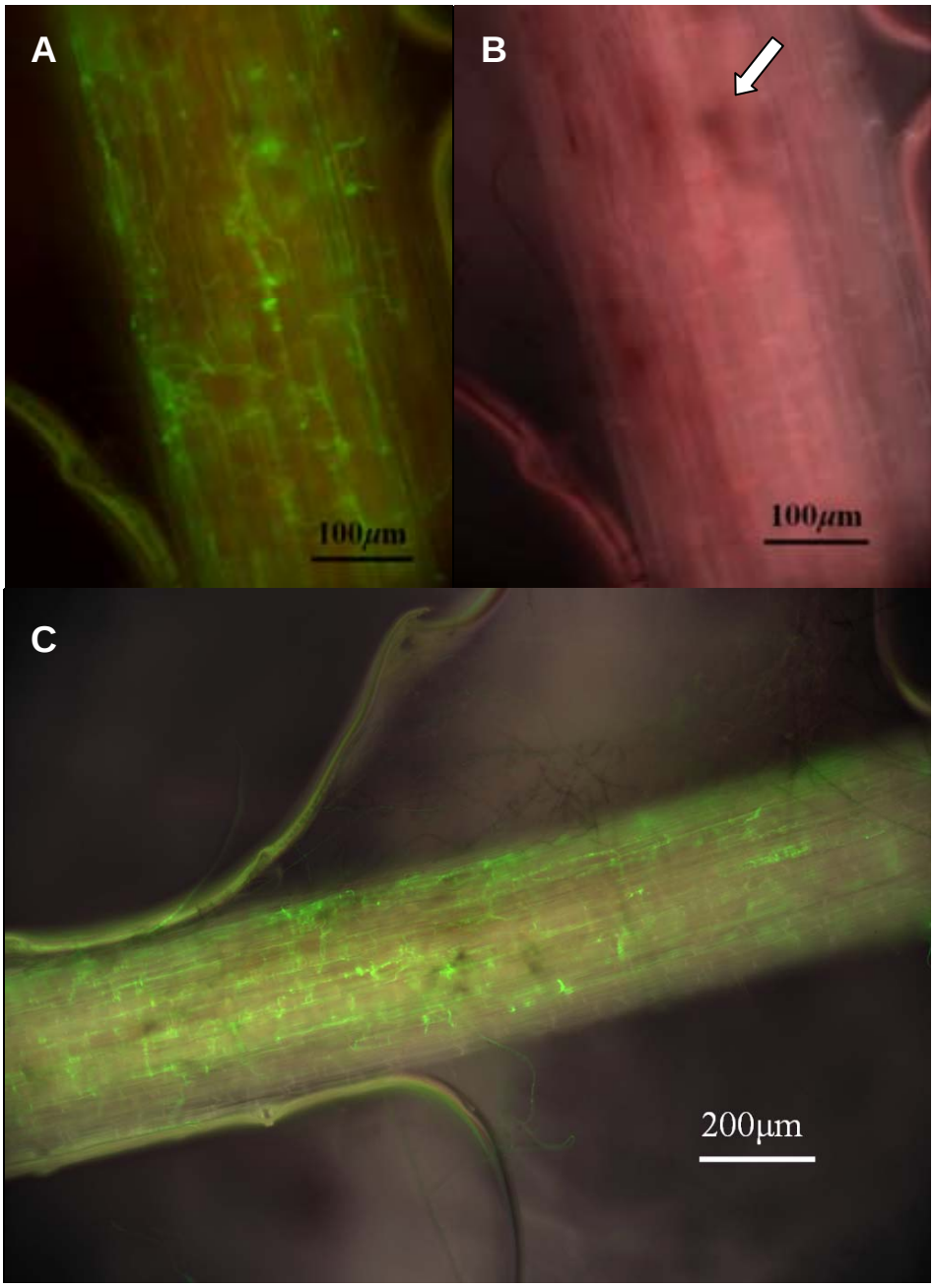

Figure 21. Root colonization and necrotic lesion formation on *Cynodon transvaalensis* at 10 days post-inoculation. A) root surface colonized by *Ophiosphaerella herpotricha* with hyphal aggregates (GFP and TRITC composite); B) root surface colonized by *O. herpotricha* with limited necrosis (arrow) (UV and TRITC composite); and C) root surface colonized by *O. herpotricha* with loose network of ectotrophic hyphae (GFP and UV composite).

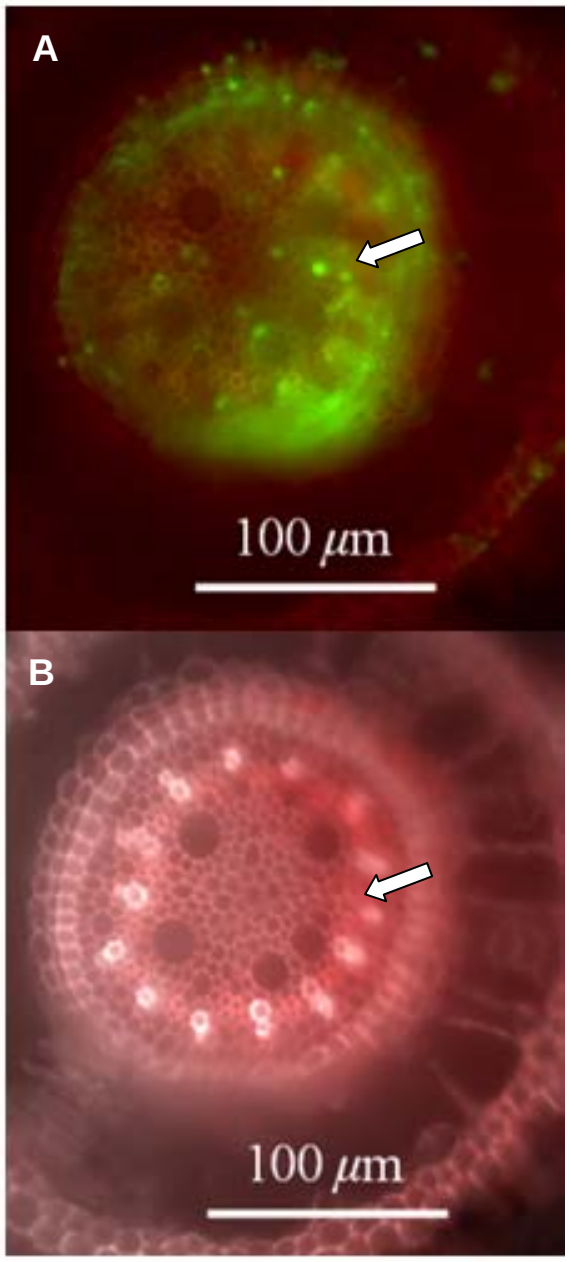

Figure 22. Transverse root sections showing colonization and necrotic lesion formation in *Cynodon transvaalensis* at 14 days post-inoculation. A) *Ophiosphaerella herpotricha* hyphae in the stele (arrow) (GFP and TRITC composite); and B) colonized stele (arrow) with no necrosis (UV and TRITC composite).

Disease severity ratings. Root disease ratings differed among Tifway 419, Midlawn and the *C. transvaalensis* accession for macroscopic, microscopic lesions and root surface colonization ratings at 14 DPI (Table 1). Tifway 419 had the highest macroscopic lesions severity ratings among the three plants. The *C. transvaalensis* accession had the lowest root lesions ratings. For all the cultivars, epi-fluorescence with the UV-filter set was used to detected microscopic lesions on root surface. There were no differences in microscopic root lesion ratings for Midlawn and Tifway 419, while *C. transvaalensis* severity ratings were lower.

Detection of tdTom signal, a measure of colonization, varied for the three grasses. Tifway 419 had the highest level of colonization. Midlawn roots were moderately colonized whereas *C. transvaalensis* had lowest level of root surface colonization at 14 DPI. Of the three plants tested, Tifway 419 had most severe lesion symptoms and root colonization, followed by Midlawn and then *C. transvaalensis*.

Transverse sections of *C. transvaalensis* roots revealed hyphae of *O. herpotricha* expressing tdTom fluorescent protein in the vascular region. Intra- and intercellular colonization of root cortical tissues for all three plants. Necrotic symptoms were most prevalent in the cortical infections of Midlawn and Tifway 419. The colonization was lowest for *C. transvaalensis* as a majority (70%) of the plants had colonization with only ectotrophic hyphae on the root surface and no hyphal aggregates. However when the level of colonization of *C. transvaalensis* was extensive, dense hyphal aggregates similar to Tifway was observed and the UV necrosis was less compared to Tifway. *Cynodon transvaalensis* roots were discolored (yellow) and had minimal tan to brown visual necrosis, whereas Tifway and Midlawn roots had dark brown visible necrotic lesions.

Overall, *C. transvaalensis* colonization by *O. herpotricha* is less dense, less aggregated, and less necrotic than Tifway 419 and Midlawn.

Table 1. Root disease severity for three turf-type bermudagrasses infected by a tdTom expressing isolate of *Ophiosphaerella herpotricha.*

Cultivar/Accession	Visible Epidermal Lesions[w]	Epidermal Lesions (UV)[y]	Epidermal colonization (TRITC)[z]
Tifway 419	3.65 a [x]	5.10 a	5.90 a
Midlawn	2.90 b	4.20 a	4.10 b
C. transvaalensis	1.40 c	2.80 b	2.90 c

[w] Observed roots visually. Based on a scale of 0 to 5, where: 0 = no root discoloration, 1 = yellow root discoloration without necrotic lesions, 2 = scattered small light tan brown lesions (<25% of root), 3 = root discoloration with scattered solid or limited dark lesions (<25% of root), 4 = root discoloration with large dark solid lesions and very visible (<50% of root), and 5 = root discoloration with necrosis on almost entire root (>50% of root).

[x] Means of 20 plants per cultivar. Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's protected least significant difference test.

[y] Observed using an epi-fluorescence microscope at 100x with UV-2E/C DAPI (330-380 nm excitation) filter. Based on a scale of 0 to 6, where: 0 = no symptoms, 2 = less severe with only small necrotic lesions covering less than 25% of the root, 4 = moderately severe with necrotic lesions covering less than 50% of the root, and 6 = extremely severe with necrotic lesions covering more than 50% of the root.

[z] Observed using an epi-fluorescence microscope at 100x with GFP-3035B (457-487 nm excitation) filter for root autofluorescence and G-2E/C TRITC (528-553 nm excitation) filter for red (tdTom) fluorescence detection. Based on a scale of 0 to 6, where: 0 = no infection or colonization, 2 = colonization with few ectotrophic hyphae on root, 4 = less aggregated and loosely interwoven hyphae covering less than 50% of the root surface, and 6 = densely interwoven and aggregated hyphae embracing more than 50% of the root surface.

Discussion

The use of fluorescent protein expressing fungi to study host pathogens interactions have been successfully used for many fungal pathosystems (Maor et al., 1998; Lorang et al., 2001; Lagopodi et al., 2002; Venard and Vaillancourt, 2007). We were able to successfully generate transgenic isolates of *O. herpotricha* with two fluorescent proteins, GFP and tdTom. Based on initial evaluations, the disease progression for transgenic isolates of *O. herpotricha* appeared similar to that for non-transgenic isolates. The observations of *O. herpotricha* infection and colonization of bermudagrass roots at the cellular level could not have been readily observed using traditional light microscopy without extensive histology. This approach also provided a method for documentation of hyphal growth, localization of infection process, and detection of root necrosis.

Previously, the infection and colonization of roots has been characterized as being similar to that for the take-all patch fungus *Gaeummanomyces graminis*. It was suggested that hyphopodia are important structures for initial infection and infection cushions are required for penetration and colonization (Asher and Shipton, 1981; Clarke and Gould, 1997; Smiley et al., 2005). However, simple or lobed hyphopodia were not observed in this study. *Ophiosphaerella herpotricha* relies on direct hyphal penetration of roots. Fusion of ectotrophic hyphae on root surfaces to form mat-like aggregates occurred but were formed at later stages of infection. After initial penetration of epidermal layer, short transverse hyphal growth was observed but cortical colonization was predominantly longitudinal or along the root axis. Smiley et al. (2005) described that *Ophiosphaerella* frequently accumulated as ectotrophic hyphae at the junction between primary and

secondary roots. This was observed in this study, however, colonization at these junctions were not the primary infection locations.

Pre-attachment to root hairs or lateral roots was not necessary to initiate infection and no differences were evident across the *Cynodon* spp. examined 2 days after inoculation. As colonization progressed on the root surface, necrotic lesions developed and were more visible and larger in Tifway 419 and Midlawn than in *C. transvaalensis*. On most occasions, *C. transvaalensis* did not appear to respond to infection by *O. herpotricha* however necrosis would later develop as did systemic yellow discoloration of the colonized root. Root necrosis is an important factor as lesions can predispose the roots to secondary fungal and bacterial infections. Measuring lesions individually was not practical as lesions could be contiguous or scattered. Therefore a rating scale was used. Tifway 419 and Midlawn appeared more susceptible than the *C. transvaalensis* accession based on visible necrosis, intensity of microscopic lesions, and degree of root epidermal colonization. Cell wall degradation and death were more predominant in Tifway 419 and Midlawn. This response may be an effort to limit the fungus by a hypersensitive response. However, the longitudinal growth of the fungus was not limited by host cell death. In Tifway 419 and Midlawn, the fungus colonized the cortical tissues but not the central cylinder of the root. In contrast, the *C. transvaalensis* accession was more tolerant to cortical colonization by *O. herpotricha*. Vascular colonization was only observed in *C. transvaalensis* which suggesting tolerance or possibly the formation of an endophytic association.

Ophiosphaerella herpotricha is a facultative saprophyte, root-invading, and ectotrophic fungus (Smiley et al., 2005). The fungus has the ability to vigorously colonize

roots without causing necrosis in early stages of infection for Midlawn and for late stages of infection on *C. transvaalensis*. Infection of Tifway 419 almost always caused necrosis. This suggests that the interspecific hybrid like Tifway 419 and *C. dactylon* cultivars like Jackpot attempt to restrict colonization and the ectotrophic growth of *O. herpotricha* with necrosis. This concept of interactive relationship between the defense mechanisms of the host and ectotrophic growth was discussed by Landschoot et al. (1993). Clearly, Tifway 419 and Midlawn to some extent showed rapid and aggressive necrotic responses to infection which could be recognized as a phytotoxic host cell death to feed a necrotroph. It was also shown that the fungus has the ability to produce numerous infection foci. Ironically, hypersensitivite cell death of these cultivars may be the cause of their susceptibility to SDS.

The more resistant *C. transvaalensis* accession's ability to tolerate colonization may be attributed to its lack of necrotic response. By not responding, it encouraged a less damaging endophytic colonization of *O. herpotricha* and avoided epidermal damage due to ectotrophic colonization. Disease severity ratings showed that necrotic lesions were smaller and fewer on *C. transvaalensis* and the majority of roots had only systemic discoloration. The roots were also soft and often easily detached from the stolons which may suggest root abscission is another form of defense mechanism.

Endo et al. (1985) described the infection of bermudagrass roots and other organs by *Ophiosphaerella korrae in planta.* They described fusiform sclerotia consisting of parallel hyphae arising internally from the endodermal tissues and appeared in the root cortex. This type of sclerotial formation was not observed for GFP expressing *O. herpotricha*. The mat-like aggregates of hyphae on the root surface appeared to have

formed externally and no sclerotia-like structures were observed in the cortex. The observation period may not have been long enough to permit sclerotial development.

This study suggests that direct root infection is predominant and varies for cultivars. Infection and colonization is rapid and within 10 to 14 days, lesions and root necrosis occurred for Tifway 419 and Midlawn. Results of this study suggest that increasing cold tolerance, possibly a trait from *C. transvaalensis* also may incorporate greater tolerance to *O. herpotricha* and less root necrosis.

This study also demonstrated the utility of using *O. herpotricha* isolates expressing fluorescent protein as an early screening method for spring dead spot. Current *in vivo* screening methods usually require 5 to 7 years whereas this rapid screening technique would only require a few weeks. This approach would reduce time and cost of screening bermudagrass genotypes and permit detection of SDS susceptibility earlier in cultivar development.

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CHAPTER III

INFECTION AND COLONIZATION OF THE STOLONS OF THREE TURF-TYPE BERMUDAGRASSES BY *OPHIOSPHAERELLA HERPOTRICHA* EXPRESSING THE RED FLUORESCENT PROTEIN, tdTOMATO

Introduction

Spring dead spot (SDS), caused by *Ophiosphaerella* spp., is the most important disease of turf-type bermudagrass species of common bermudagrass (*Cynodon dactylon* (L.) Pers.) and the interspecific hybrid cultivars (*Cynodon dactylon* x *C. transvaalensis* Burtt-Davy) in North America and Australia (Smith, 1971; Lucas, 1980; Endo et al., 1985; Taliaferro et al., 2003; Smiley et al., 2005). Turf-type bermudagrass cultivars used for residential and institutional lawns, athletic fields, parks, and golf courses in regions where grass is dormant during the winter are commonly affected by SDS (Smiley et al., 2005). Cultivars such as Tifway 419 or Tifsport are popular commercial hybrids that are affected by SDS particularly when the stand is intensively managed and three years or older. (Lucas, 1980; Pair et al. 1994; McCarty and Miller, 2002; Smiley et al., 2005).

Ophiosphaerella herpotricha is the predominant causal agent of SDS in the Midwestern region of North America (Clarke and Gould, 1997; Wetzel et al., 1999a; Smiley et al., 2005). Two other fungi identified as causal agents of SDS are *O. korrae*

and *O. narmari* (Smith, 1971; Endo et al., 1985; Wetzel et al., 1999b; Smiley et al., 2005). In the past, SDS was associated with *Helminthosporium* and other root-rotting fungi including *Gaeumannomyces graminis* (Smith, 1965; Smith, 1971; McCarty and Lucas, 1989, Smiley et al., 2005). Since it was first reported 50 years ago, there is still not a clear understanding of the host-pathogen interaction and disease progression (Tisserat et al., 1989, Baird et al., 1998; Smiley et al., 2005; Walker et al., 2006).

Smiley et al. (2005) and Clarke and Gould (1997) described fungal structures present on field collected root and stolon surfaces such as sclerotia, infection mats, and pseudothecia but were not able to directly describe the infection and colonization by *Ophiosphaerella* spp. inside the root and stolon tissues. Smiley et al. (2005) described the *Ophiosphaerella* spp. as fungi with dark brown, septate mycelia, which form sclerotia on roots and stolons. Smiley et al. (2005) also mentioned the occasional presence of pseudothecia on infected stolons. Crahay et al. (1988) observed pseudothecia on infected bermudagrass roots as black, flask-shaped, with a thick neck. However, they did not report sclerotia or sclerotia-like bodies.

Ophiosphaerella are ectotrophic and root invading fungi, that cause diseases of both warm and cool season grasses (Smiley et al., 2005). Techniques such as transforming fungi with fluorescence markers using *Agrobacterium tumefaciens* and observing host-plant interactions has been used in various pathosystems such as tomato root rot, citrus black rot, rice blast, maize anthracnose, and alfalfa spring black stem (Lagopodi et al., 2002; Ishiki et al. 2003; Sesma and Osbourn, 2004; Dhulipala, 2007; Venard and Vaillancourt, 2007).

The objective of this research was to describe stolon infection and colonization of bermudagrass turf hybrid cultivars Midlawn, and Tifway 419 (*Cynodon dactylon* var. *dactylon* x *Cyndon transvaalensis* Burtt-Davy) and an accession of African bermudagrass (*Cynodon transvaalensis*) by *O. herpotricha*.

Materials and Methods

Propagation and inoculation of bermudagrass stolons. Plants were propagated and stolons inoculated as described previously with the exception that stolons were directly inoculated with agar plug (approximately 0.25 mm in diameter) containing mycelium of *O. herpotricha* expressing red (tdTom) fluorescent protein. This study was repeated once.

Microscopy. Microscopic observations were conducted using a Nikon Eclipse E-800 epi-fluorescent microscope. Stolons were observed at 3, 14 and 28 days post-inoculation (DPI). Root-inoculated plants from Chapter II were observed 4 weeks post-inoculation. Stolons were embedded in 3% agarose and hand-sectioned with surgical blade (Bard-Parker, Becton Dickinson Acute Care, Franklin Lake, NJ). Stolon surfaces, transverse sections, and longitudinal sections were observed using an epi-fluorescence microscope equipped with mercury-vapor lamp as light source and set of filters including G-2E/C TRITC (EX 528-553 nm/EM 600-660 nm) for red (tdtom) fluorescence detection, UV-2E/C DAPI (EX 330-380 nm/EM 435-485 nm) for UV autofluorescence and necrosis detection, and GFP-3035B (EX 457-487 nm/EM 503-538 nm) for stolon autofluorescence. Digital images were generated as described in Chapter II.

Results

Tifway 419 stolons. Epi-fluorescence microscopic examination of internode-inoculated stolons revealed ectotrophic hyphae on the stolon surface of Tifway 419 at 3 DPI (Fig. 23A). Transverse sections did not indicate penetration of epidermis; however ectotrophic hyphae were present on the surface at 3 DPI (Figs. 23B, 23C). Loose hyphal aggregates were observed on stolon surfaces along with numerous ectotrophic hyphae at 3 DPI (Fig. 23C). At 14 DPI, necrotic lesions on stolons were colonized by surface hyphae and hyphal aggregates were present at site of inoculation (Figs. 24A, 24B). Composite images showed aggregates of hyphae or infection mats corresponded with necrotic lesion (Fig. 24B). Transverse sections of stolons contained no hyphae 14 DPI (Fig. 24C). Aggregates of hyphae on the stolon surfaces 28 DPI were also observed (Figs. 25A-C). A loosely interwoven network of hyphae appeared attached to the stolon surface and vaguely resembled sclerotia but were relatively less compact and smaller (10 µm) (Fig. 25B). Slight necrosis at the point of attachment was also present (Fig. 25C).

Stolons of root-inoculated plants were also observed. After 4 weeks, longitudinal sections of stolon revealed that the plants were heavily colonized with numerous hyphae growing along the necrotic hollow center of the stolons after entering through the wound created by cutting stolons into segments 6 or more weeks earlier (Fig. 26, A and B). The fungus also colonized the cells adjacent to the hollow center and appeared to be growing intercellularly, however it did not appear to result in necrotic response.

Midlawn stolons. Epi-fluorescence microscopic examination of internode-inoculated stolons revealed few ectotrophic hyphae of *O. herpotricha* on the stolon at 3 DPI (Fig. 27A). Longitudinal stolon sections suggested no penetration of epidermal layer

at 3 DPI (Fig. 27B). At 14 DPI, the stolon surfaces of Midlawn were colonized at the site of inoculation by ectotrophic hyphae and loose hyphal aggregates (Fig. 28A). Stolons contained necrotic areas on the stolon surface with corresponding aggregates of hyphae at 14 DPI (Fig. 28B). At 28 DPI, stolons of Midlawn were not extensively colonized but had aggregates of hyphae on the surface (Fig. 29A). The infection caused necrotic lesions associated with the aggregates of hyphae (Fig. 29B) but surface colonization was not as extensive as those interwoven networks of hyphae on Tifway 419 stolons. Compact aggregates of hyphae on Midlawn stolons at 28 DPI resulted in lesions on the stolon surface (Figs. 29, C and D) and in transverse sections (Figs. 30, A and B) hyphae were observed penetrating the epidermal layer of the stolon and causing cell death.

Hollow necrotic centers of stolons of root-inoculated Midlawn plants after one month were colonized by the fungus (Figs. 31A, 31B). Hyphae appeared to enter through the wound created, 6 weeks prior, where the stolon was cut. The fungus appeared to grow intercellularly towards healthy cells around the hollow center. Extensive colonization of the hollow center and cortical layers were observed at the wall (Fig. 31B). The cells adjacent to the hollow center were less colonized and less necrotic than those of Tifway.

***C. transvaalensis* stolons**. At 3 DPI, ectotrophic hyphae were present on the stolon at the site of inoculation (Fig. 32A). Transverse and longitudinal sections suggested no penetration at 3 DPI but ectotrophic hyphae were loosely attached on the stolon surface (Figs. 32B-D). At 14 DPI, few ectotrophic hyphae were found on the surface (Fig. 33A) and longitudinal sections showed no indication of hyphal penetration (Fig. 33B). At 28 DPI, more extensive colonization was observed with ectotrophic hyphae on the stolon surface (Fig. 34A) and loose hyphal aggregate attached to the

surface (Fig. 34C). Surface colonization was less aggregated and less necrotic than Tifway 419 and Midlawn. Observations of transverse sections revealed that epidermal layer of stolons were free from infection after 28 days (Fig. 34B).

Stolons of root-inoculated *C. transvaalensis* plants were found to be extensively colonized 4 weeks post-inoculation. Longitudinal sections of stolon revealed numerous hyphae in the outer and inner tissues of the stolon and the absence of a hollow or necrotic stolon center (Fig. 35). Intercellular longitudinal colonization of the stolon tissues was predominant and hyphae grew toward the node (Fig. 35, A and B). Overall, the colonization of *C. transvaalensis* stolon tissues by *O. herpotricha* did not result in necrosis.

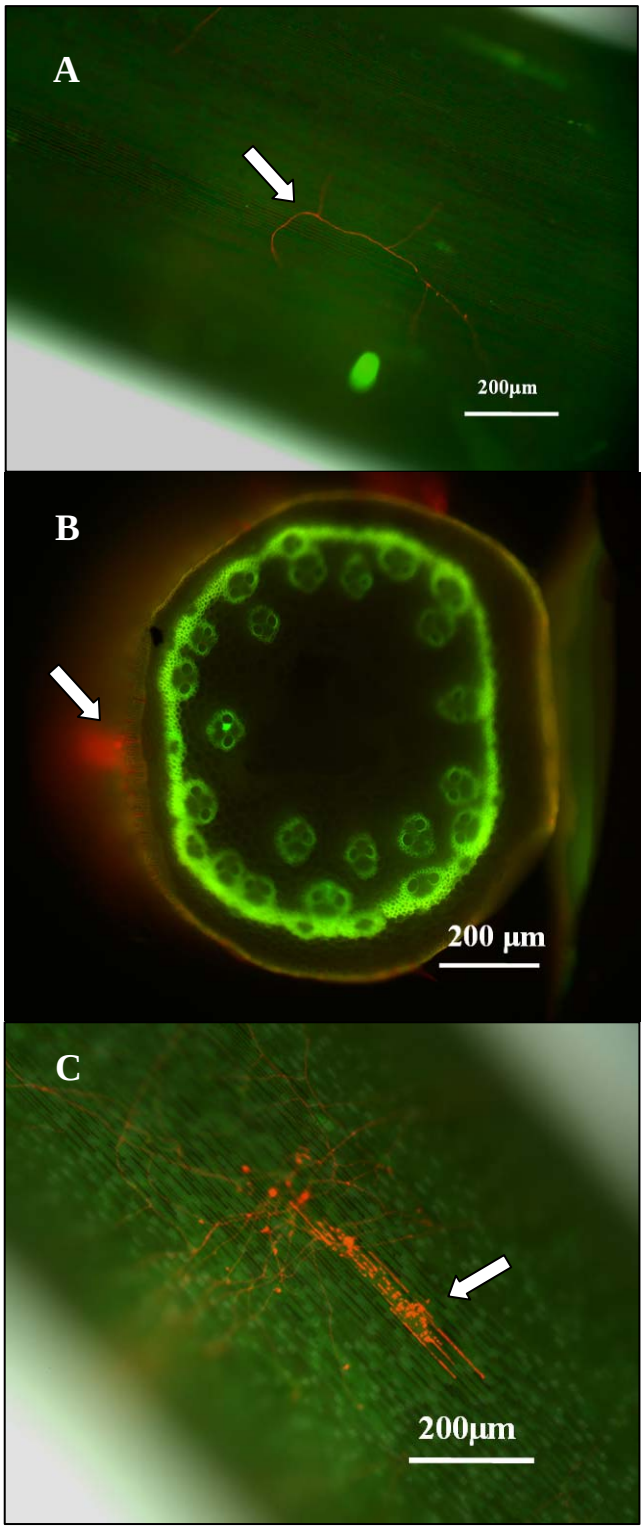

Figure 23. *Ophiosphaerella herpotricha* on Tifway 419 stolon 3 days post-inoculation.
A) ectotrophic hyphae (arrow) on stolon; B) transverse section of stolon with ectotrophic hyphae (arrow) on surface; and C) stolon surface with numerous ectotrophic hyphae and loose hyphal aggregates (arrow).

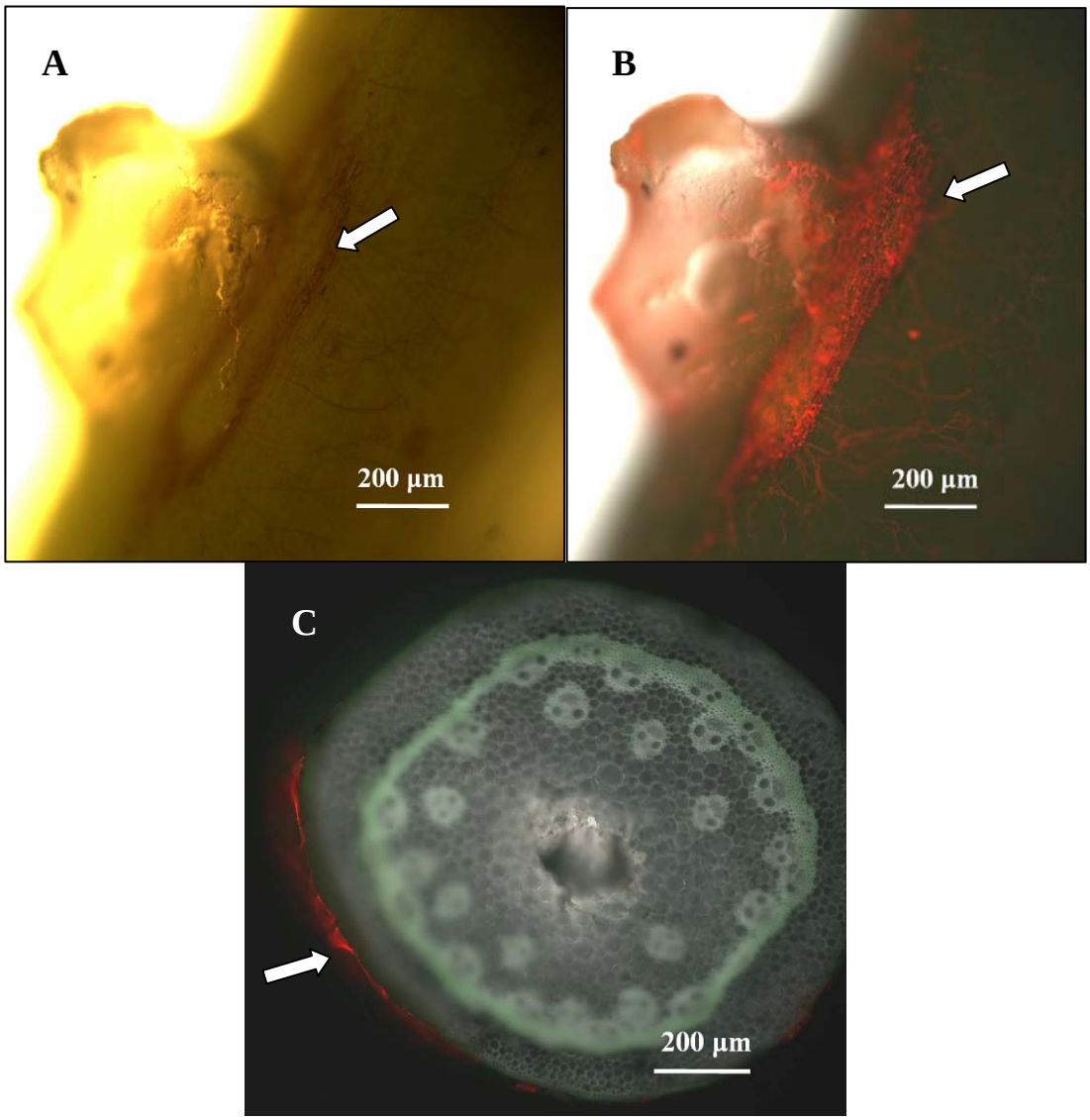

Figure 24. *Ophiosphaerella herpotricha* on Tifway 419 stolon 14 days post-inoculation. A) stolon surface with visible necrosis near the agar plug with mycelium; B) aggregates of hyphae (arrow) fluorescing red on stolon surface; and C) transverse section of stolon with ectotrophic hyphae (arrow).

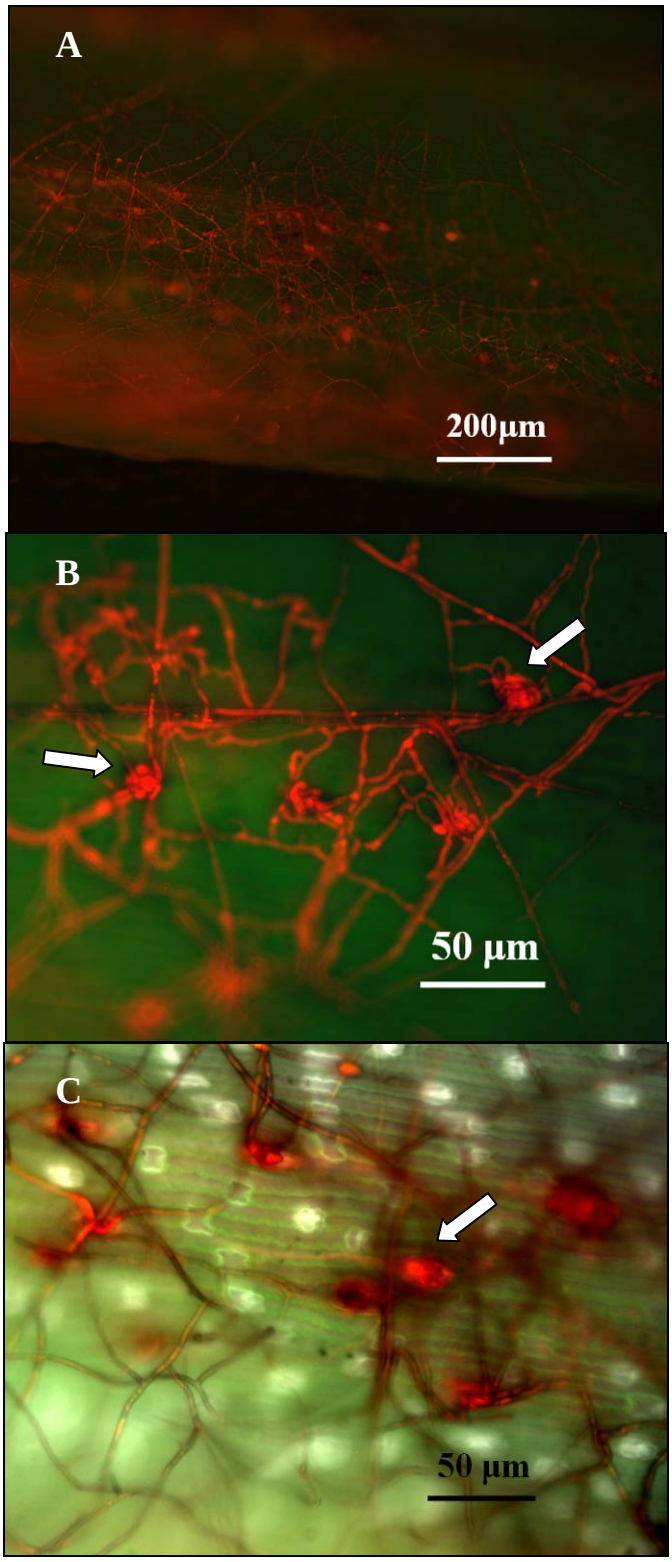

Figure 25. *Ophiosphaerella herpotricha* on Tifway 419 stolon 28 days post-inoculation. A) aggregates of hyphae on stolon; B) aggregates of hyphae (arrows) forming on stolon; and C) loose aggregates of hyphae (arrow).

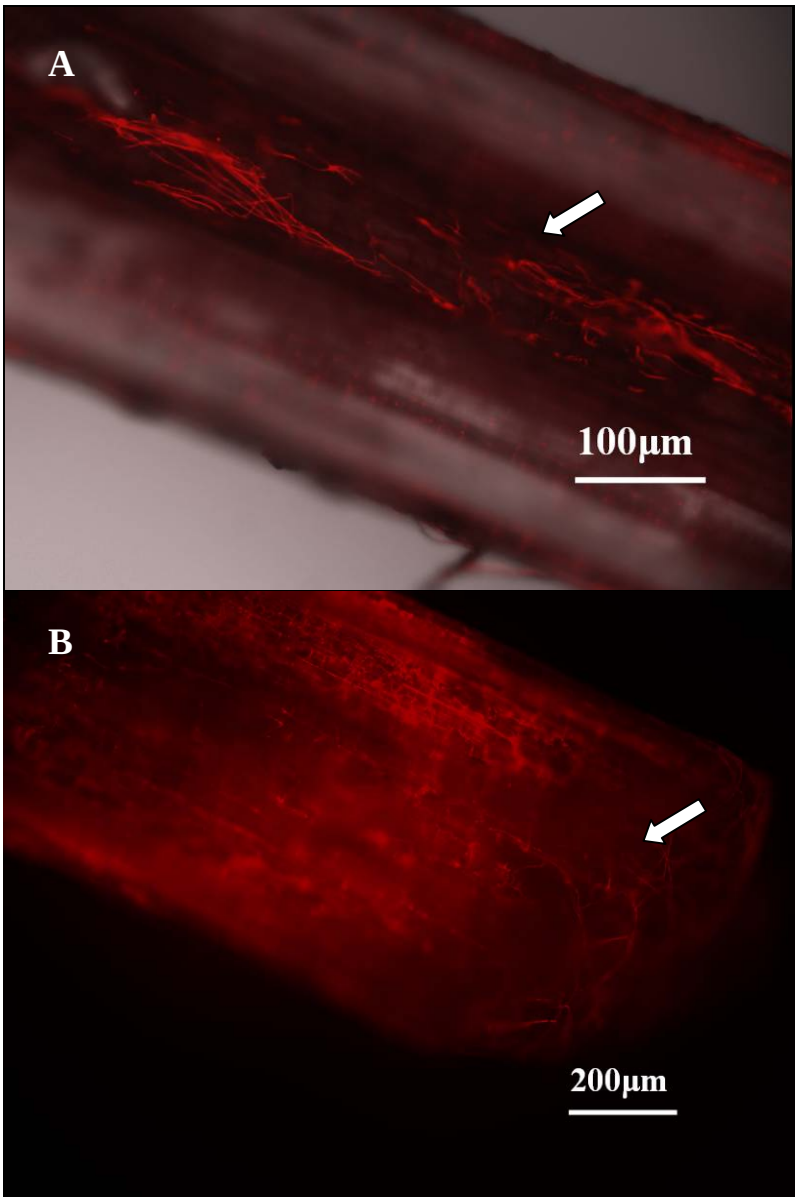

Figure 26. Root-inoculated Tifway 419 stolons containing *Ophiosphaerella herpotricha* at 4 weeks post-inoculation. A) longitudinal section of stolon with hyphae growing along the hollow center (arrow) after entering through a wound; and B) hyphae entering through the callus (arrow) and colonizing adjacent cells 4 weeks after root inoculation.

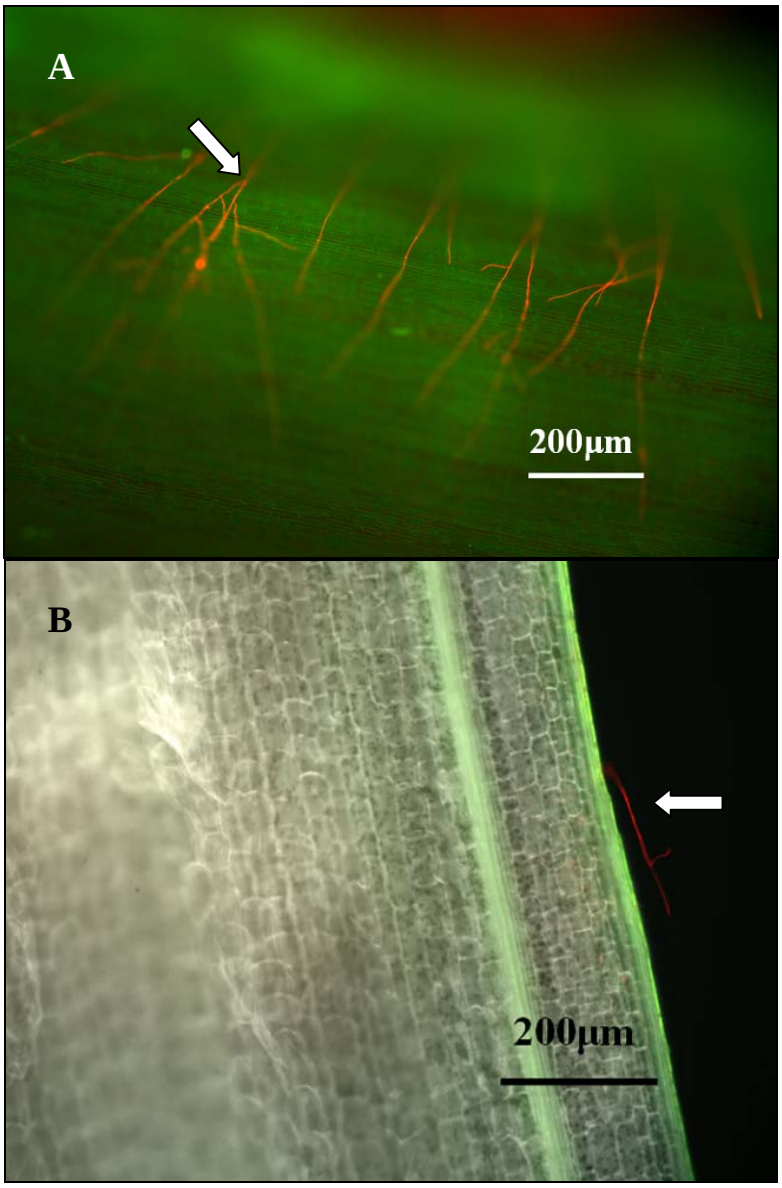

Figure 27. *Ophiosphaerella herpotricha* on Midlawn stolon 3 days post-inoculation. A) ectotrophic hyphae (arrow) on the stolon surface; and B) longitudinal section of stolon with ectotrophic hyphae (arrow) on epidermal layer.

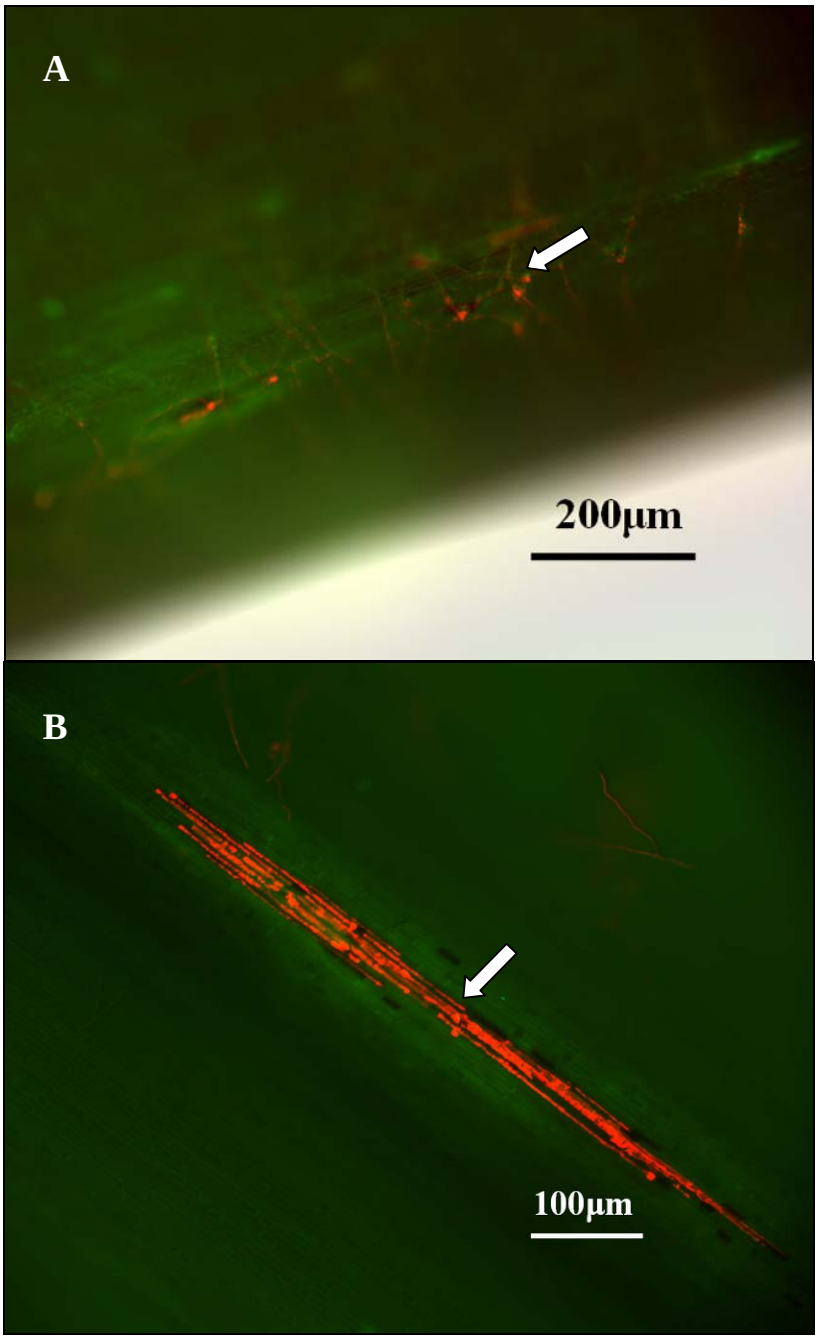

Figure 28. *Ophiosphaerella herpotricha* on Midlawn stolon 14 days post-inoculation. A) stolon surface with ectotrophic hyphae and loose hyphal aggregates (arrow); and B) stolon surface with an aggregate of hyphae (arrow).

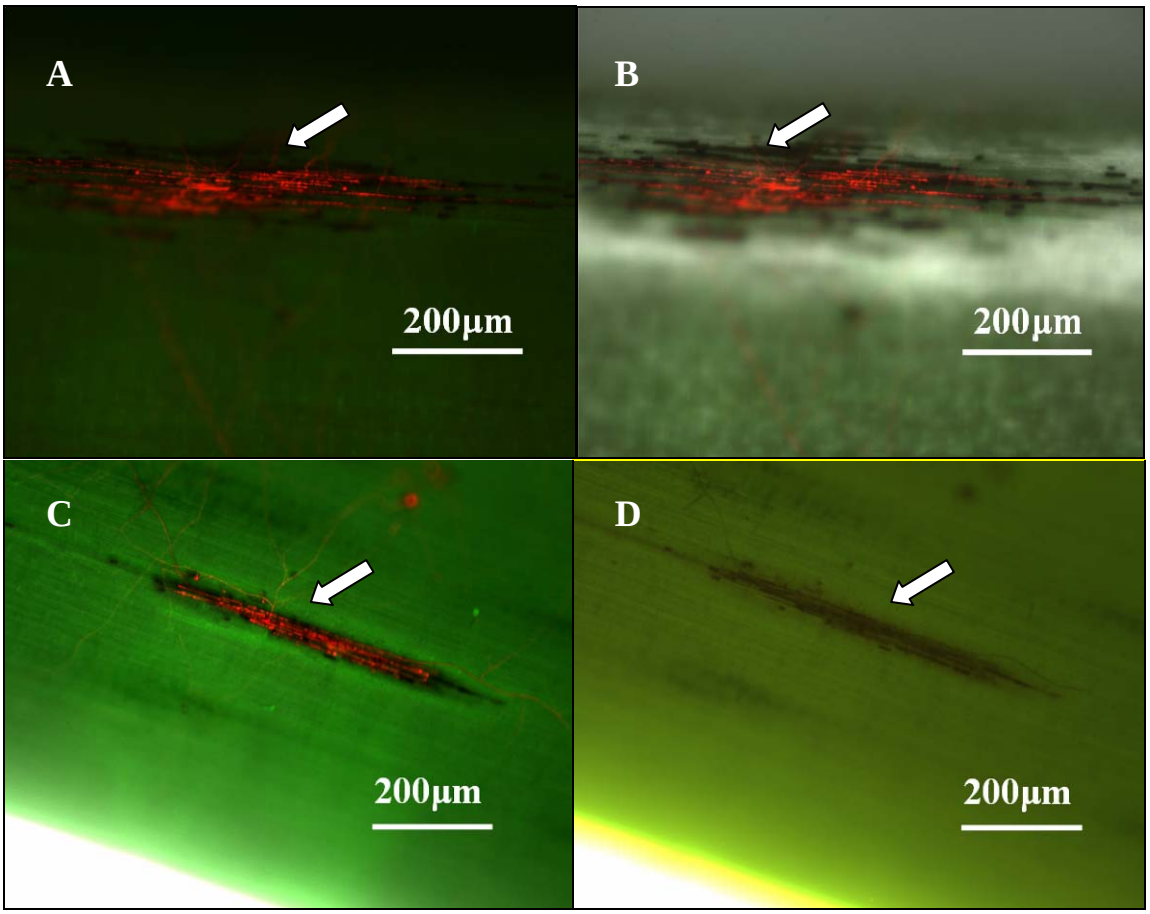

Figure 29. *Ophiosphaerella herpotricha* on Midlawn stolon 28 days post-inoculation. A) stolon surface with aggregates of hyphae (arrow); B) expanding necrosis (arrow) underneath the aggregates of hyphae; C) compact aggregates of hyphae (arrow) on stolon; and D) visual necrosis on stolon (arrow).

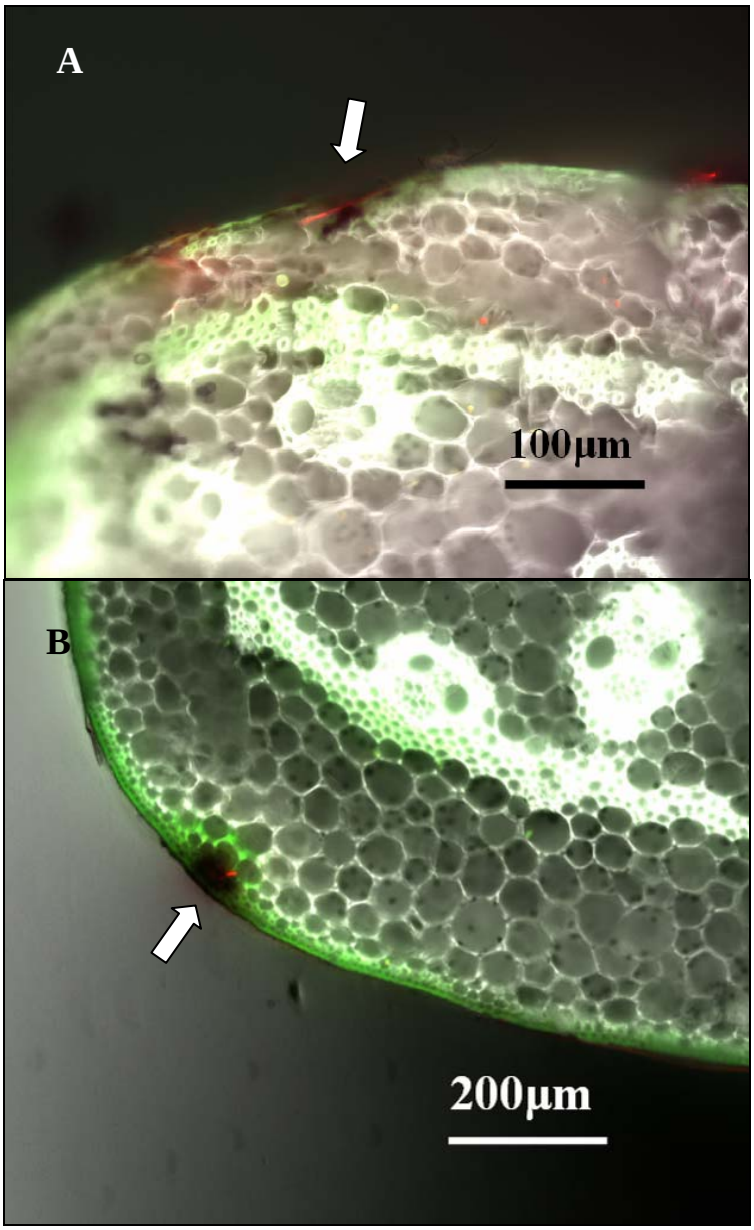

Figure 30. Transverse section of Midlawn stolon penetrated by *Ophiosphaerella herpotricha* 28 days post-inoculation. A) transverse section showing epidermal layer (arrow) of stolon penetrated; and B) transverse section of stolon with hyphae (arrow) penetrating the epidermal layer.

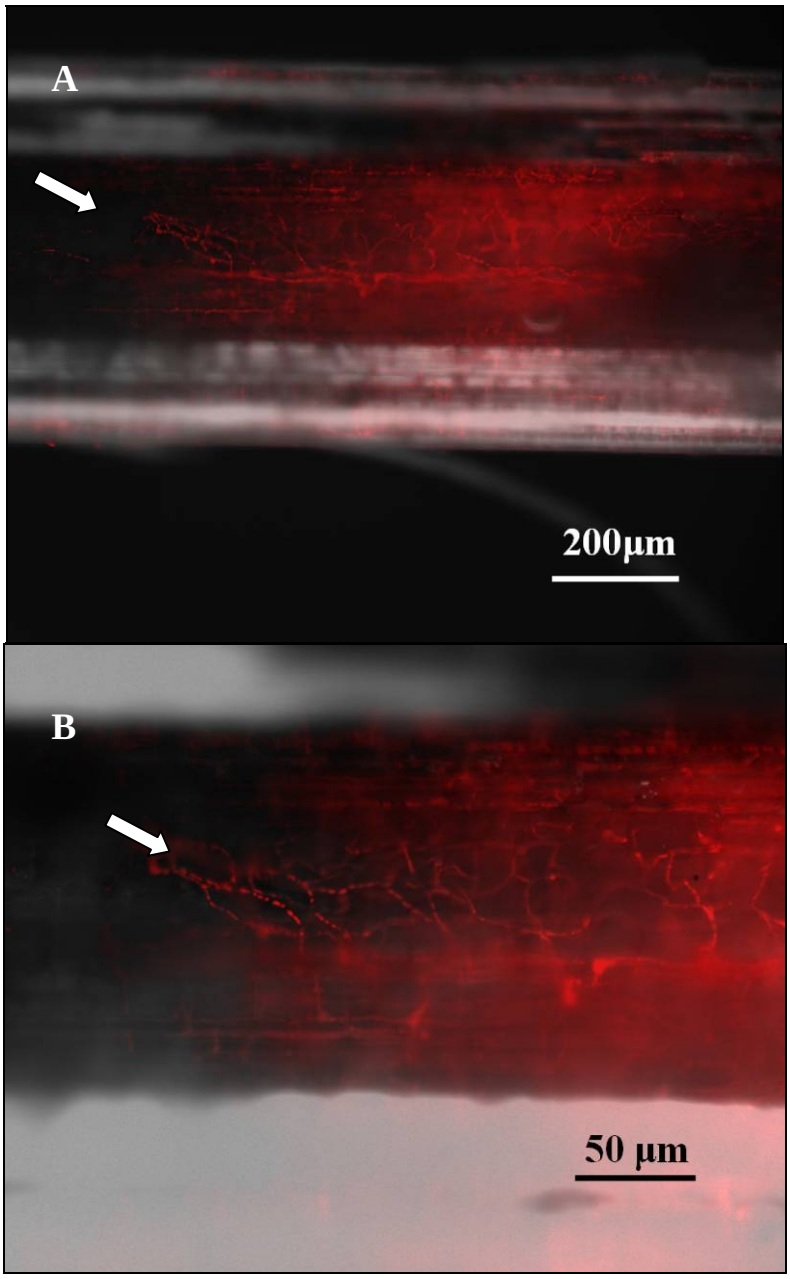

Figure 31. Root-inoculated Midlawn stolons containing *Ophiosphaerella herpotricha* at 4 weeks post-inoculation. A) hyphae growing through the hollow center (arrow) of the stolon; and B) hollow center of stolon colonized by *O. herpotricha* (arrow) after entering through a wound.

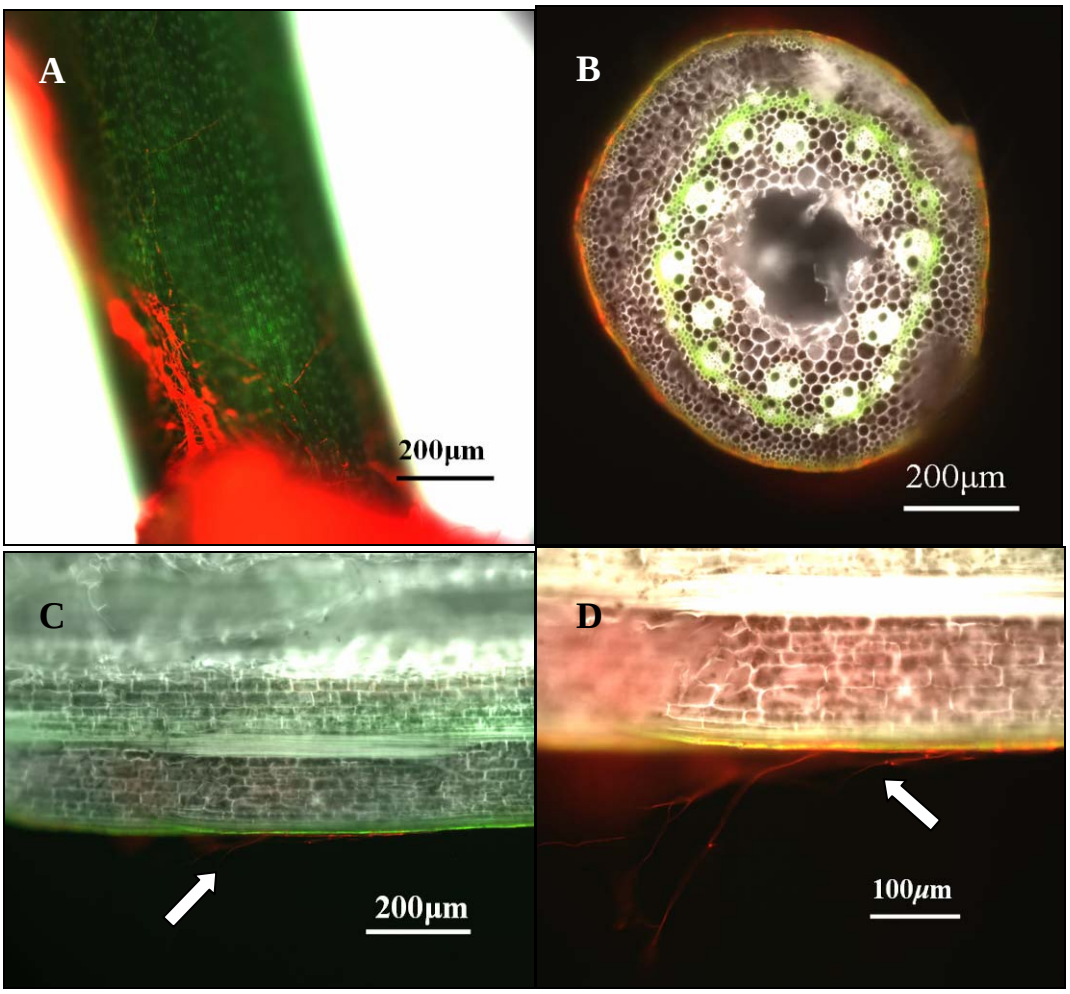

Figure 32. *Ophiosphaerella herpotricha* on *Cynodon transvaalensis* stolon 3 days post-inoculation. A) ectotrophic hyphae embracing the stolon; B)transverse section of stolon with hyphae on surface; C) longitudinal section of stolon with ectotrophic hyphae (arrow) on surface layer; and D) longitudinal section of stolon with ectotrophic hyphae (arrow) on surface.

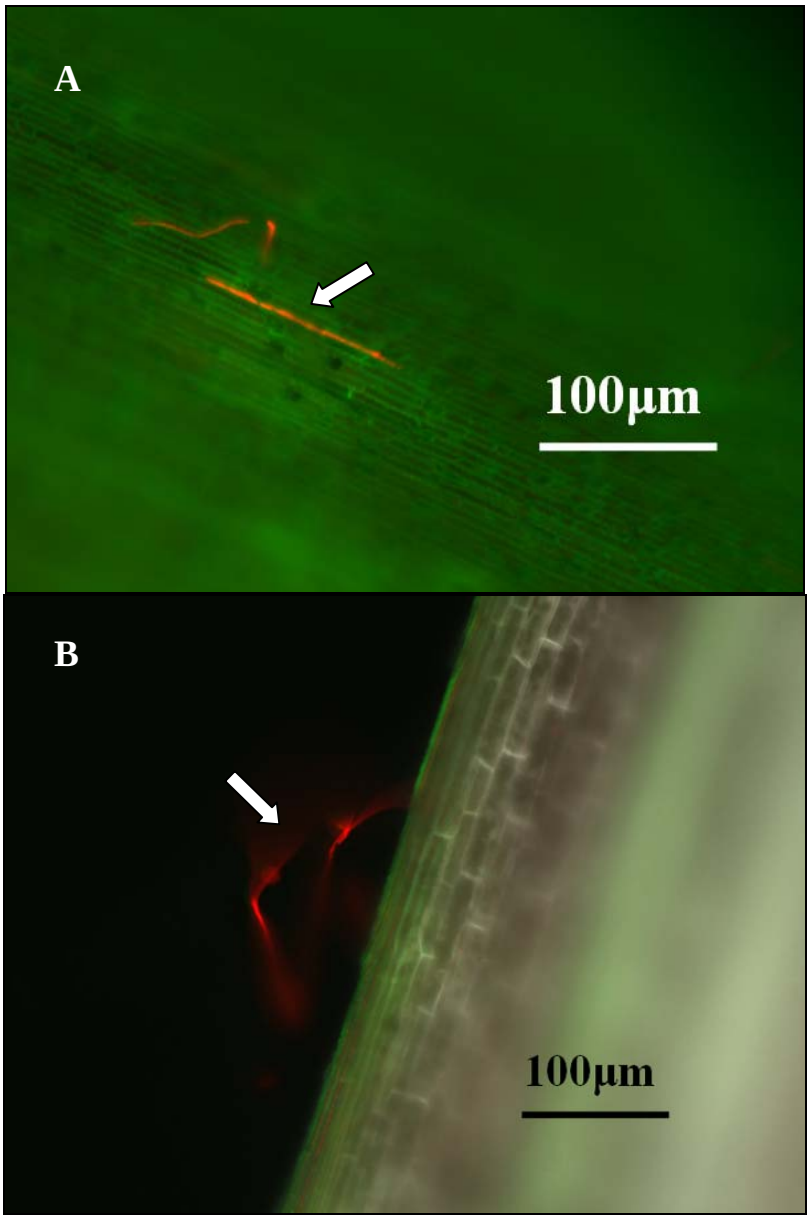

Figure 33. *Ophiosphaerella herpotricha* on *Cynodon transvaalensis* stolon 14 days post-inoculation. A) stolon surface with ectotrophic hyphae (arrow); and B) longitudinal section with ectotrophic hyphae (arrow).

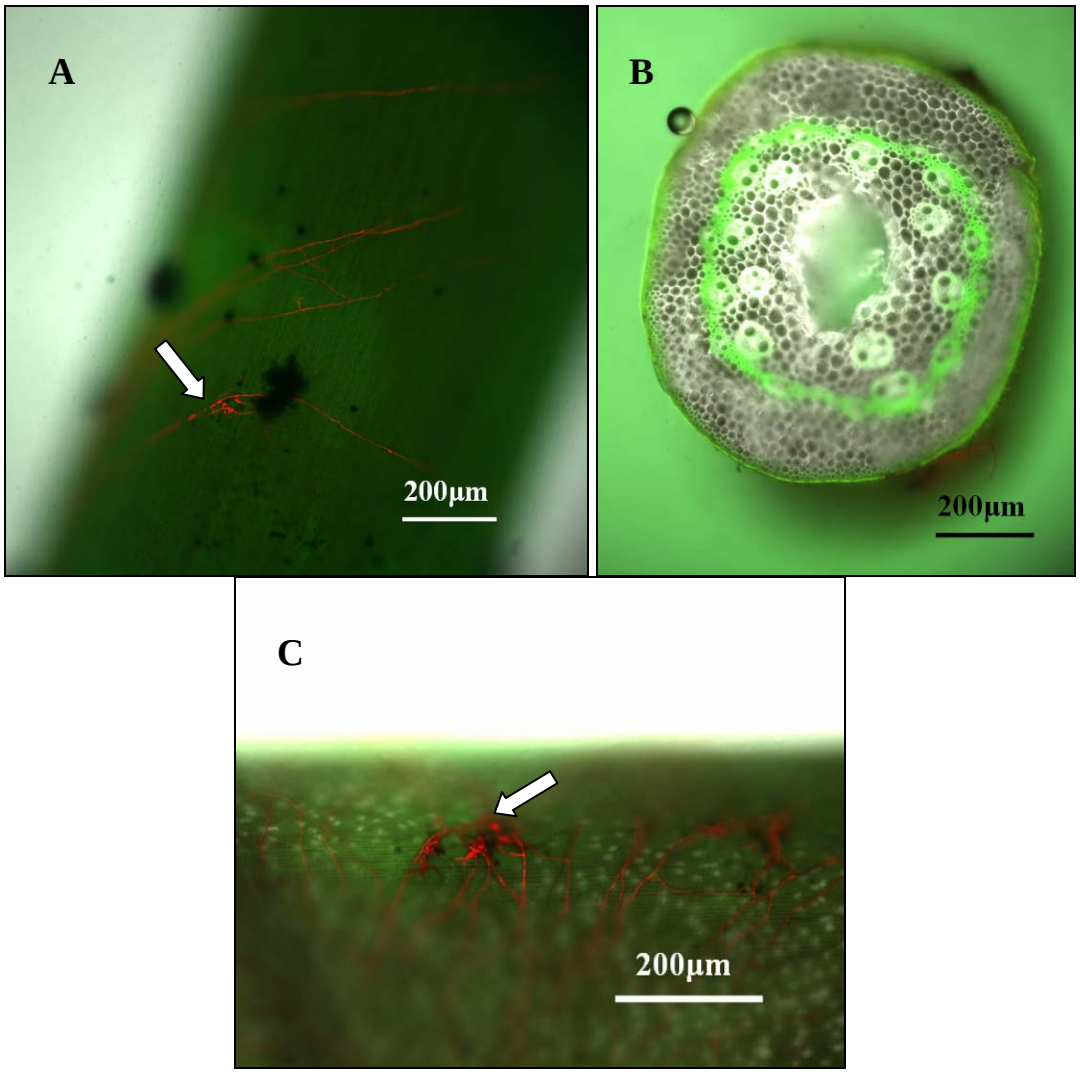

Figure 34. *Ophiosphaerella herpotricha* on *Cynodon transvaalensis* stolon 28 days post-inoculation. A) hyphae (arrow) on the stolon surface and loose hyphal aggregates; B) transverse section showing epidermal layer of stolon with no infection; and C) aggregates of hyphae (arrow) on the stolon surface with no visible necrotic lesion.

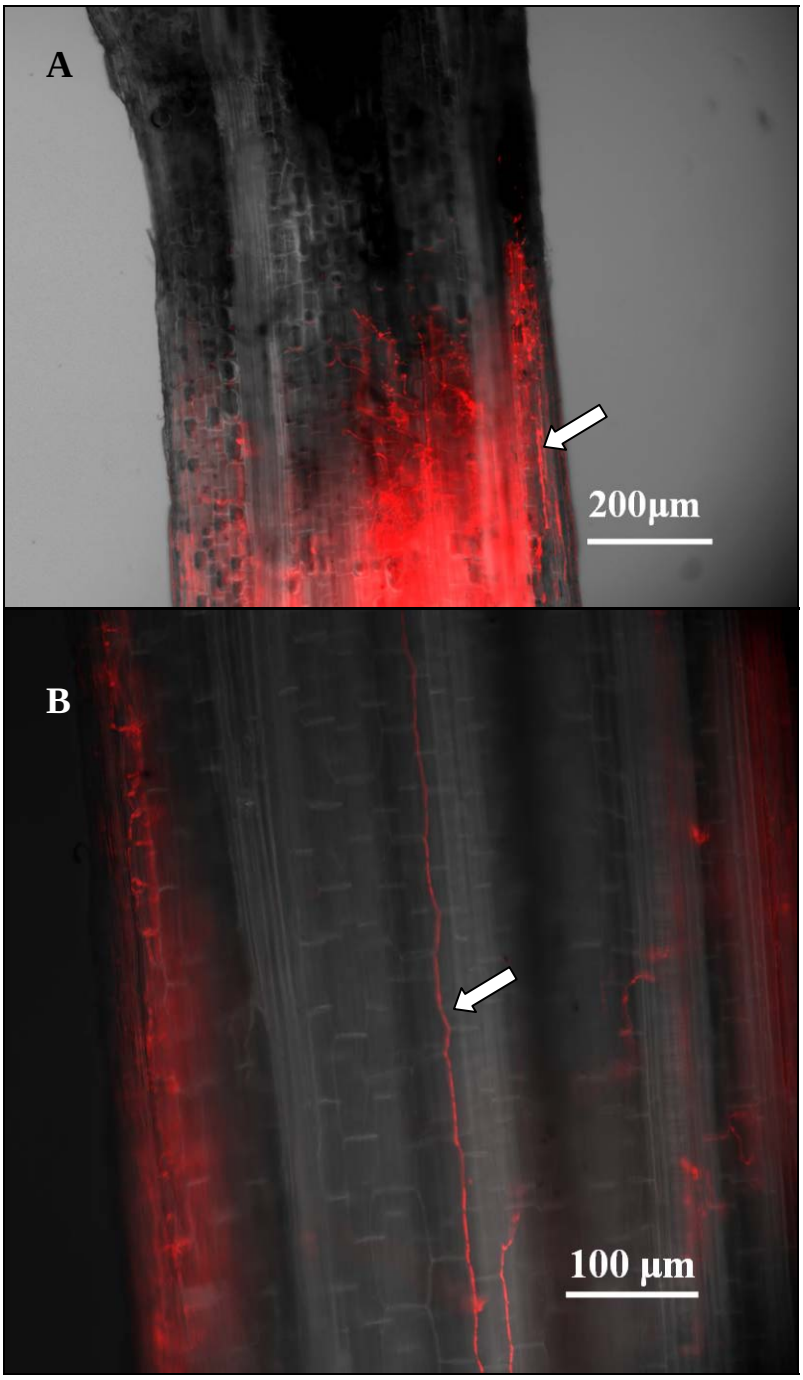

Figure 35. Root-inoculated *Cynodon transvaalensis* stolons containing *Ophiosphaerella herpotricha* at 4 weeks post-inoculation. A) longitudinal section of stolon with hyphae (arrow) extensively colonizing the outer and inner tissues of the stolon; and B) intercellular colonization (arrow) of the stolon tissues after entering through a wound.

Discussion

This is the first study to directly examine stolon infection by *Ophiosphaerella herpotricha*. This study demonstrated the significance of stolon infection as a possible alternative infection site for spring dead spot (SDS) development. Based on initial evaluations, transgenic isolates were similar to wild type in infection and colonization rate. *Ophiosphaerella herpotricha* can slowly and less efficiently penetrate the stolon epidermis and may colonize the plant through this process. The fungus may prefer to enter through the root tissues but this study revealed that ectotrophic hyphae can extensively colonize the inner stolon tissues through healed wounds. For the SDS fungus, stolons could potentially provide better nutritional source and longer survival through better protection from environmental conditions.

The fungus was capable of endophytic movement in lateral and longitudinal directions, branching off and colonizing the stolon's vascular region. The fungus was also able to penetrate the stolon without special structures such as hyphopodia and infection cushions. Sclerotia or mat-like structures were observed at the point of attachment. According to Smiley and others (2005), pseudothecia occasionally develop on stolon and roots but these were not observed in this study even at 28 days after stolon inoculation. Instead, stroma or infection mats (200-400 µm) were found on Tifway 419 and Midlawn but absent on *C. transvaalensis*.

More extensive colonization and hyphal aggregates were observed on Tifway 419 stolons. Visible necrotic lesions were also observed for Tifway 419 and Midlawn. It was also demonstrated here that *O. herpotricha* can enter stolons through healed wounds. In turf, stolon wounds may be caused by chewing insects, mowing or aerification and

recreational activities in home lawns and golf courses. For Tifway 419 and Midlawn, the hollow necrotic center of stolons that were colonized by *O. herpotricha* facilitated the colonization of non-necrotic cells. For *C. transvaalensis*, the center of the stolon was not hollow or necrotic but nonetheless colonized by *O. herpotricha* by intercellular movement. The presence of endophytic growth of *O. herpotricha* in stolons of Tifway, Midlawn, and *C. transvaalensis* accession suggests an overwintering capability of the fungus in stolons.

Endo et al. (1984; 1985) described signs of *Ophiosphaerella korrae*, the cause of SDS of bermudagrass in California, as brown sclerotia on stolons, rhizomes, culm bases and roots, which were associated with cell death of affected organs. Elongated sclerotia were found in the cortex of roots. In this study, sclerotia-like structures (Fig. 25B) were observed on Tifway stolon but none were found internally. For Tifway and Midlawn, the presence of hyphal aggregates on stolon's surfaces resulted in necrotic lesions. These lesions could predispose the organ to secondary infections.

The results of this study suggest that infection of stolons can occur and may play a significant role in causing plant death over winter months. If the fungus is able to colonize the stolon and cause significant necrosis as seen with Midlawn and Tifway, then this would disrupt the normal process of carbohydrate storage within the plant. This factor combined with the possibility that stolons and rhizomes could be secondarily colonized by soil microbes may explain how the plants succumb to cold temperatures and die over the winter months.

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APPENDICES

Table A-1. Nutrient stock solutions formula

A. Macronutrient stock solutions (amount in g for 100 ml)

KNO_3	10.11
$Ca(NO_3)_2$	23.62
$NH_4H_2PO_4$	5.75
$MgSO_4$	6.02

B. Micronutrient stock solutions (amount in mg for 100 ml)

KCl	74.55
H_3BO_3	309.15
$MnCl_2$ (anhydrous)	125.84
$ZnSO_4$ $7H_2O$	57.51
$CuSO_4$ $5H_2O$	12.48
$(NH_4)_6Mo_7O_{24}$	9.89

C. Sprint 330 4.02 g -100ml

D. $Na_2O_3Si_9H_20$ 2.84 g -100ml

E. For preparation of 8 L aeroponic solution, the following amount of each stock are required:

Concentrated stock solution	Amount (ml) to add to aeroponic solution
$Na_2O_3Si_9H_20$	8
KNO_3	16
$Ca(NO_3)_2$	12
$NH_4H_2PO_4$	8
$MgSO_4$	12
Micronutrient	8
Sprint 330	8

Check the pH value, and adjust to pH 5.7 with HCl.

Figure A-1. Bermudagrass plant in Petri plate lined with wet paper towel prior to inoculation.

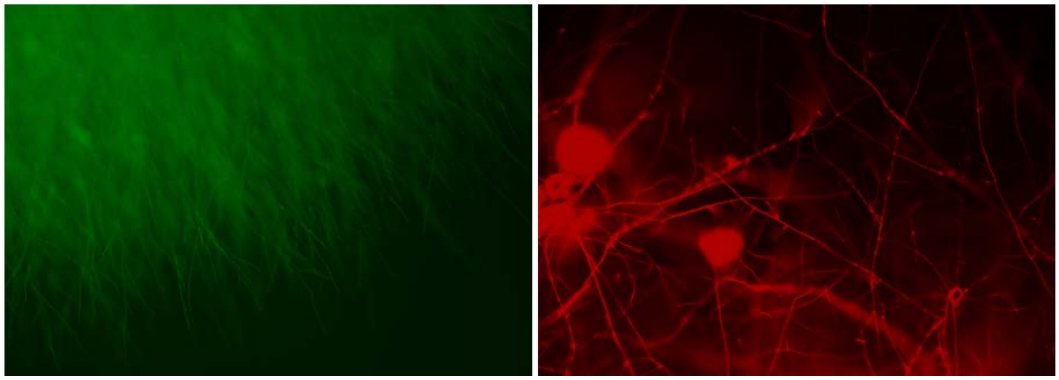

Figure A-2. *Ophiosphaerella herpotricha* transformants expressing green (GFP) (left) and red (tdTom) (right) fluorescent proteins.

Figure A-3. An isolate of *Ophiosphaerella herpotricha* transformant with tdTom fluorescent protein on YPS agar plate.

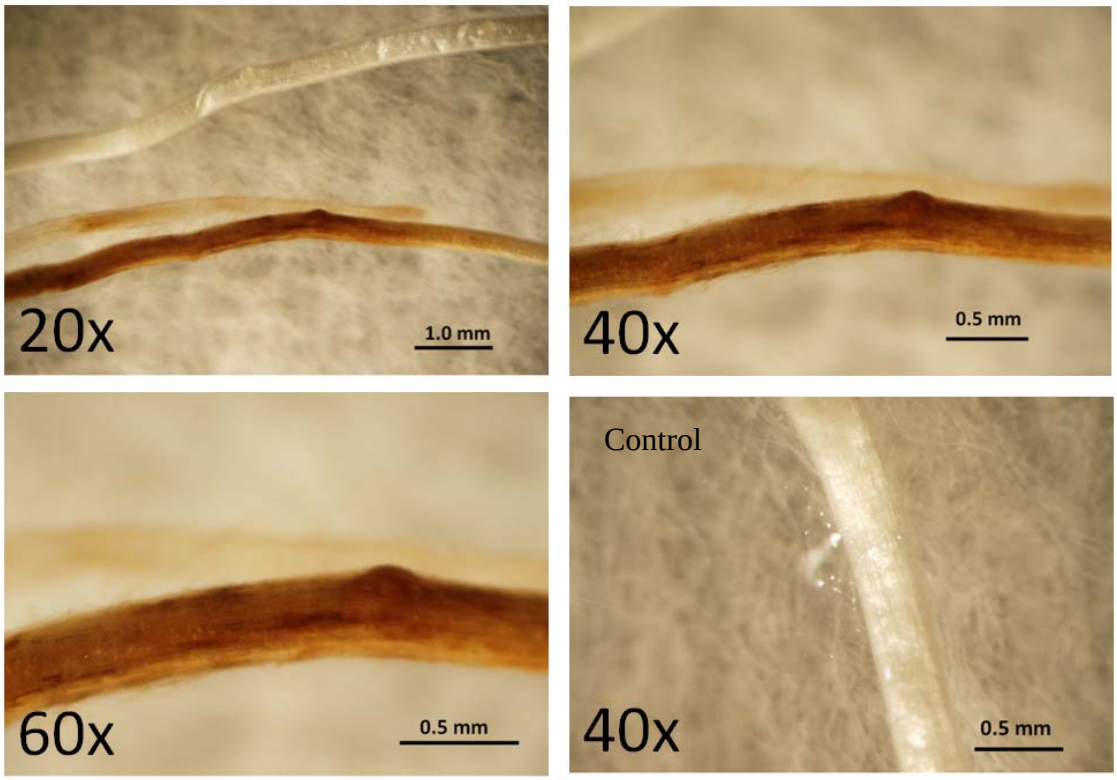

Figure A-4. Tifway 419 roots on Petri plate lined with wet paper towel 14 days post-inoculation.

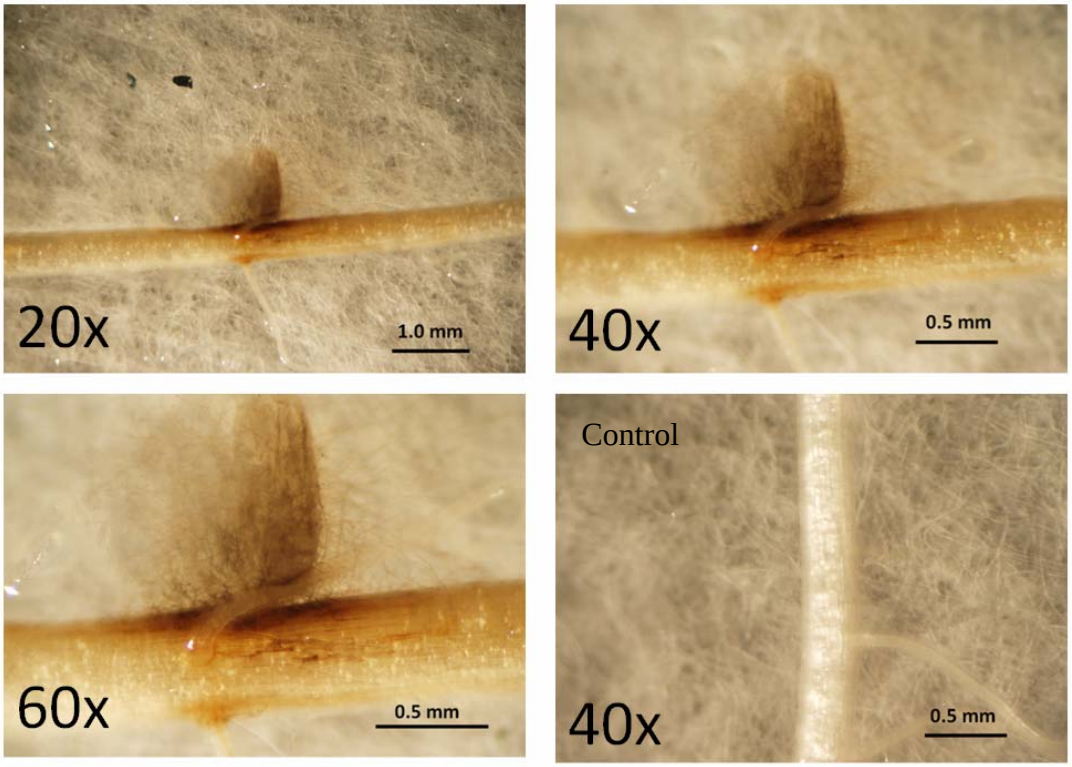

Figure A-5. Midlawn roots on Petri plate lined with wet paper towel 14 days post-inoculation.

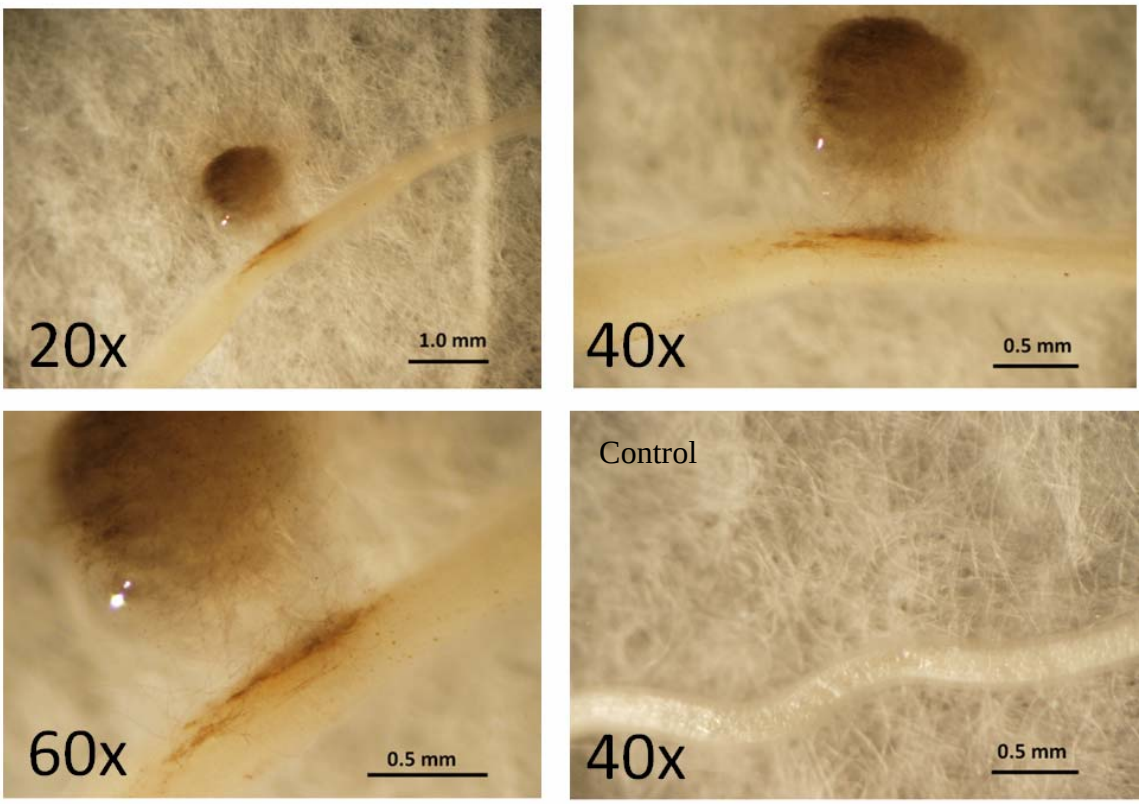

Figure A-6. *Cynodon transvaalensis* roots on Petri plate lined with wet paper towel 14 days post-inoculation.

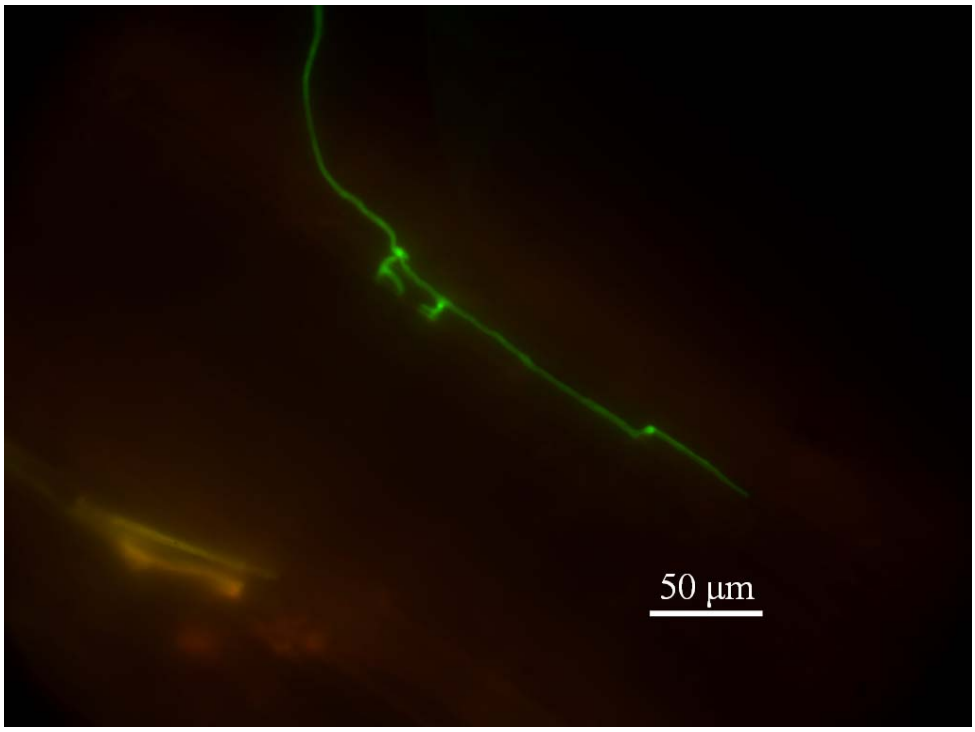

Figure A-7. Tifway 419 root colonized by GFP expressing isolate of *Ophiosphaerella herpotricha* 2 days post-inoculation (25 °C, 12 h light).

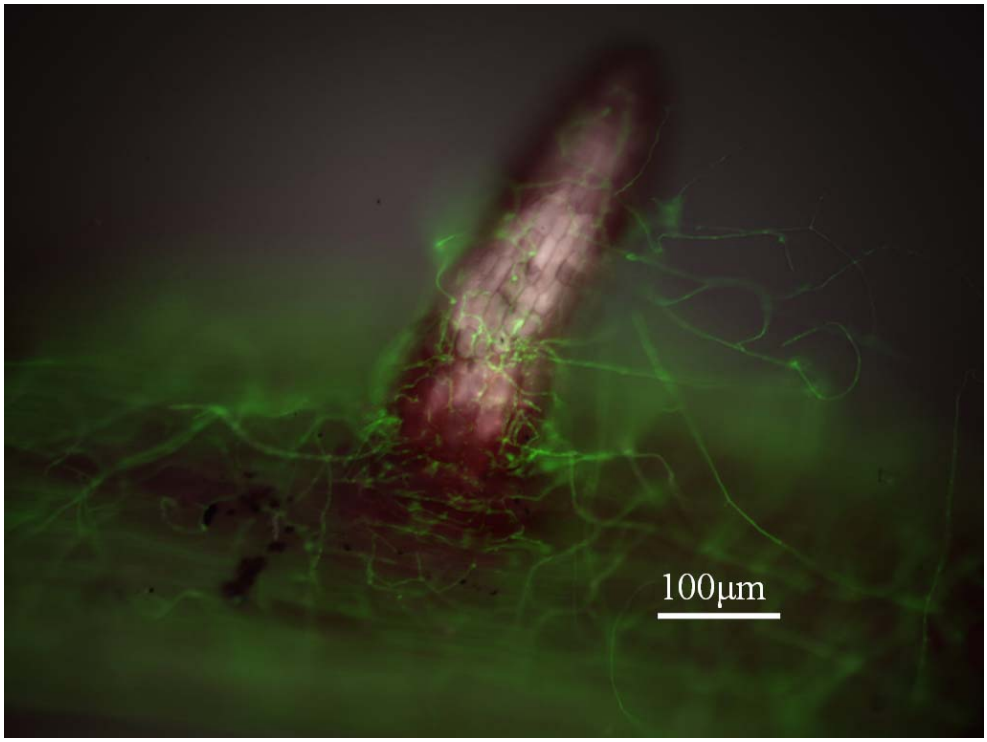

Figure A-8. Tifway 419 root colonized by GFP expressing isolate of *Ophiosphaerella herpotricha* 6 days post-inoculation (17 °C, 12 h light).

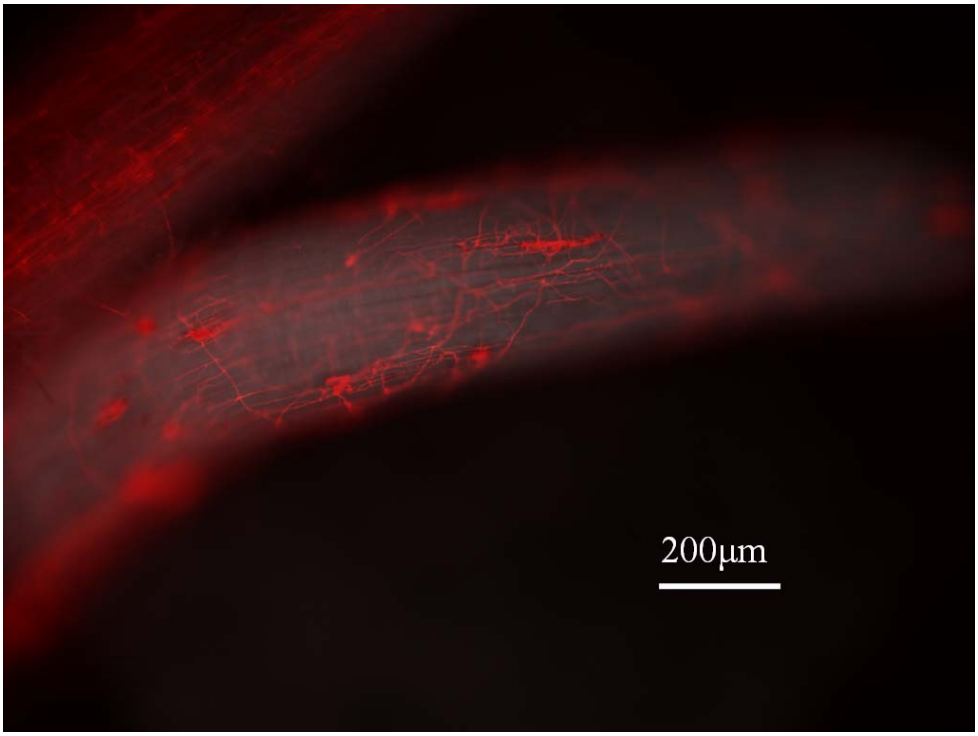

Figure A-9. Tifway 419 roots colonized by tdTom expressing isolate of *Ophiosphaerella herpotricha* at 8 DPI (17°C, 12 h light).

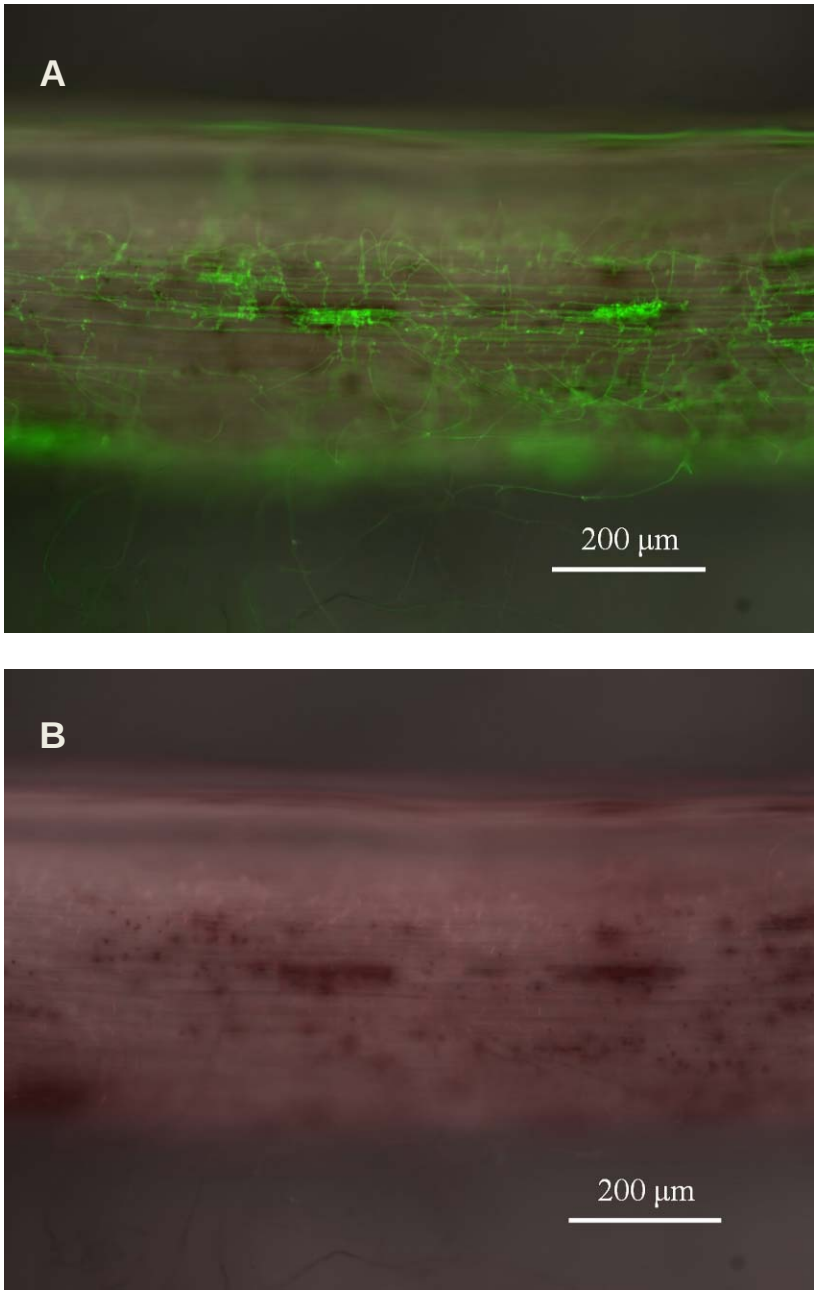

Figure A-10. Mat-like aggregates of *Ophiosphaerella herpotricha* hyphae (A) and necrotic lesions (B) on root surface of Tifway 419 at 10 DPI (17 °C, 12 h light).

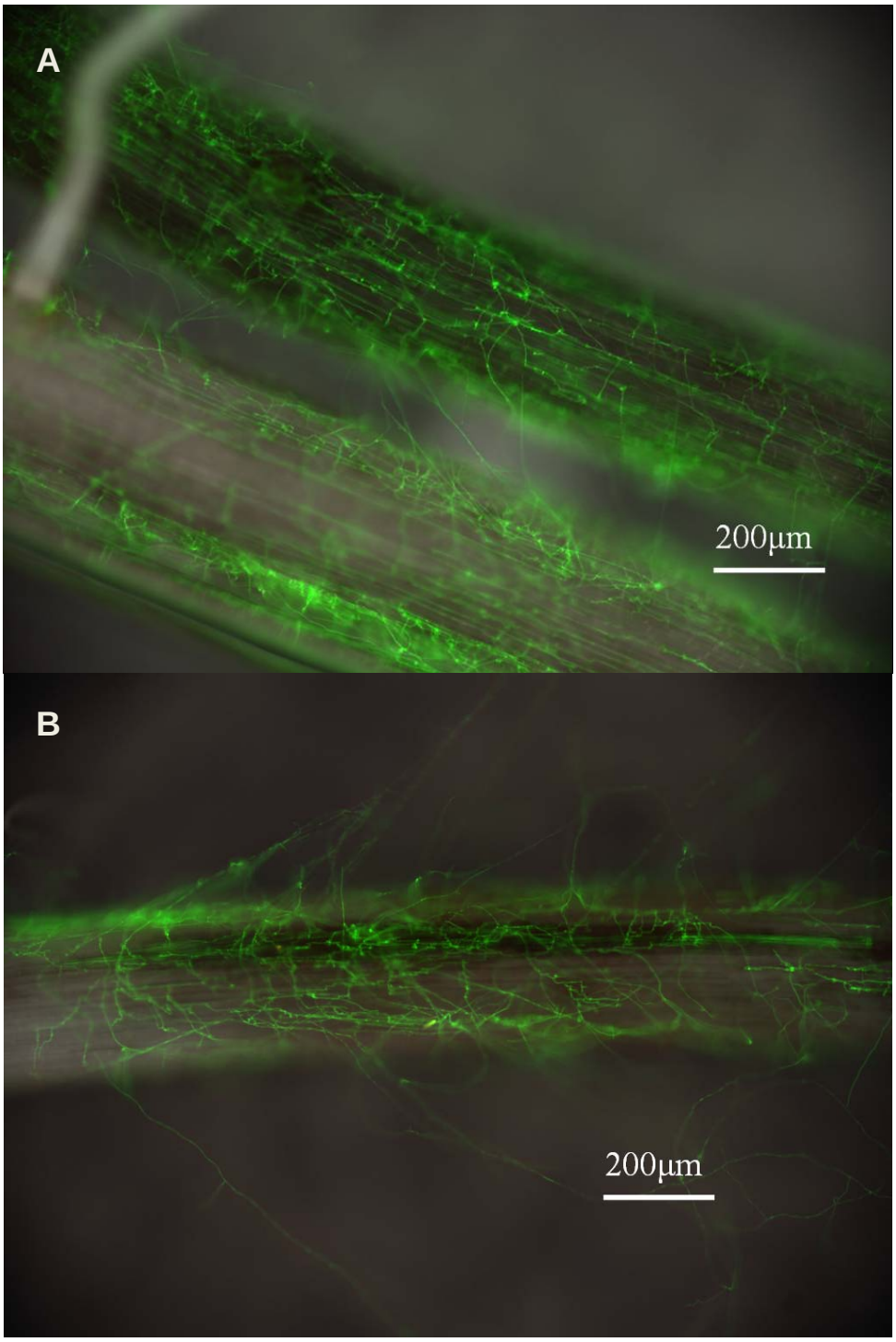

Figure A-11. Tifway 419 roots colonized by GFP expressing isolate of *Ophiosphaerella herpotricha* at 10 DPI. A) colonized roots of Tifway incubated at 17°C, 12 h light; and B) colonized root of Tifway incubated at 25°C, 12 h light.

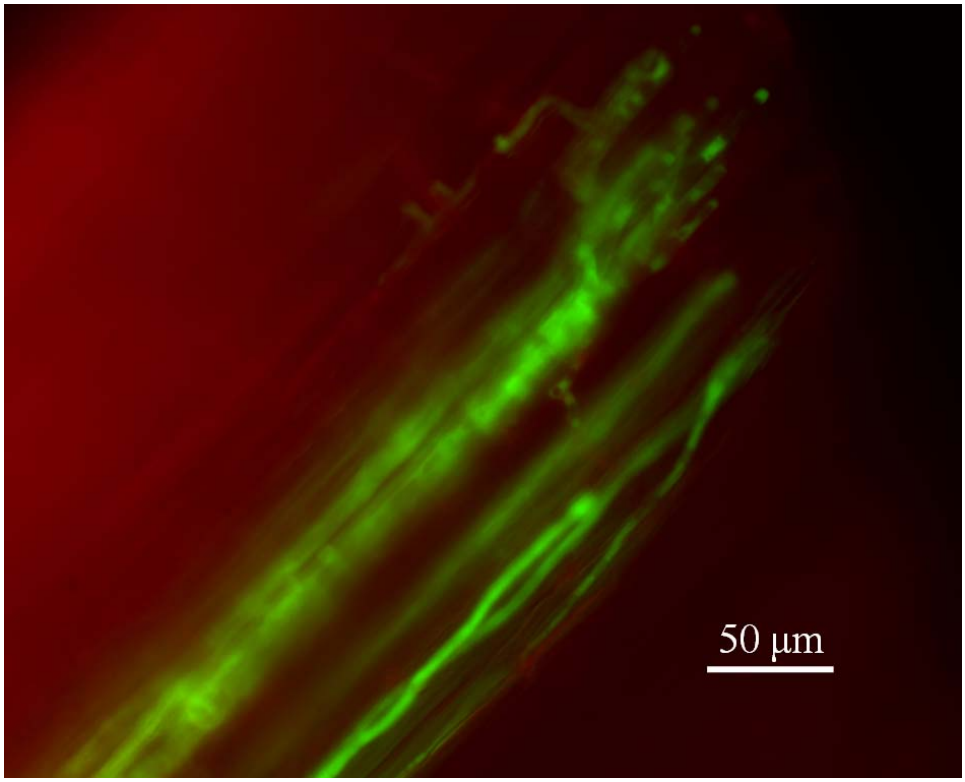

Figure A-12. Longitudinal section of Tifway 419 containing *Ophiosphaerella herpotricha* hyphae in the cortical layer at 12 DPI (17 °C, 12 h light).

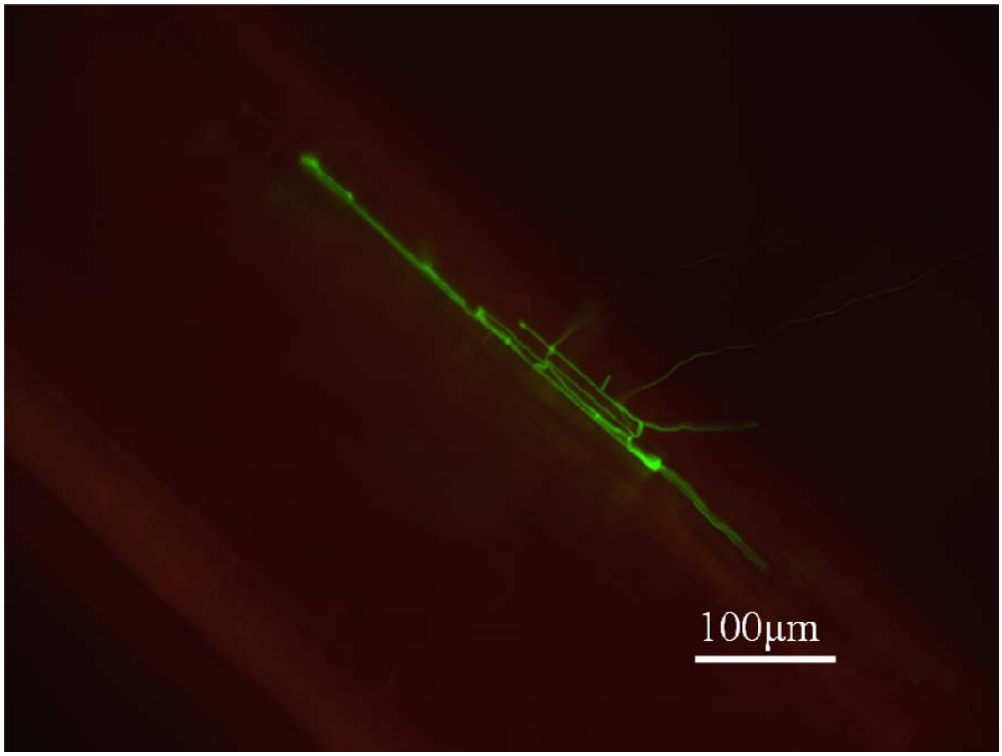

Figure A-13. Midlawn root colonized by GFP expressing isolate of *Ophiosphaerella herpotricha* at 3 DPI (17 °C, 12 h light).

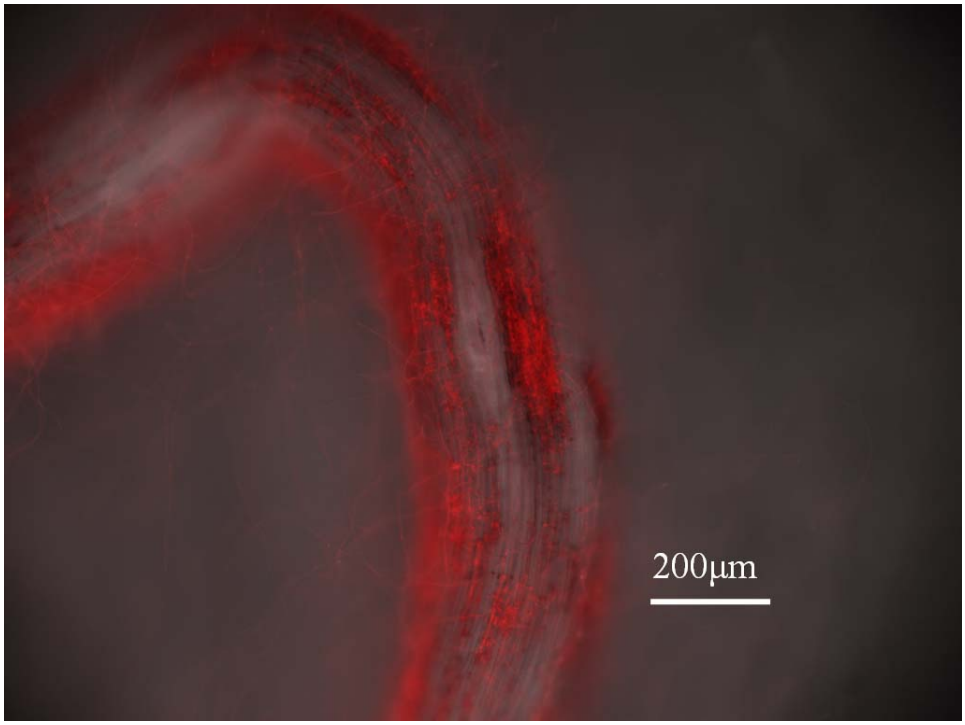

Figure A-14. Midlawn root colonized by tdTom expressing isolate of *Ophiosphaerella herpotricha* at 8 DPI (17 °C, 12 h light).

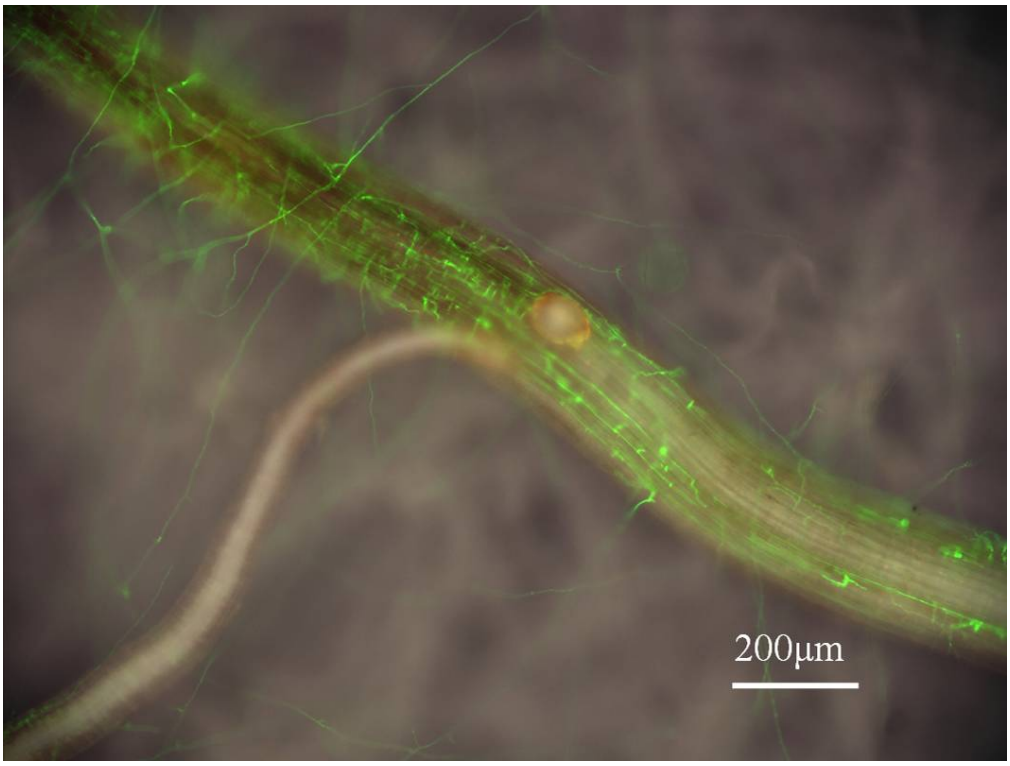

Figure A-15. Midlawn root colonized by GFP expressing isolate of *Ophiosphaerella herpotricha* after 10-day incubation (25 °C, 12 h light).

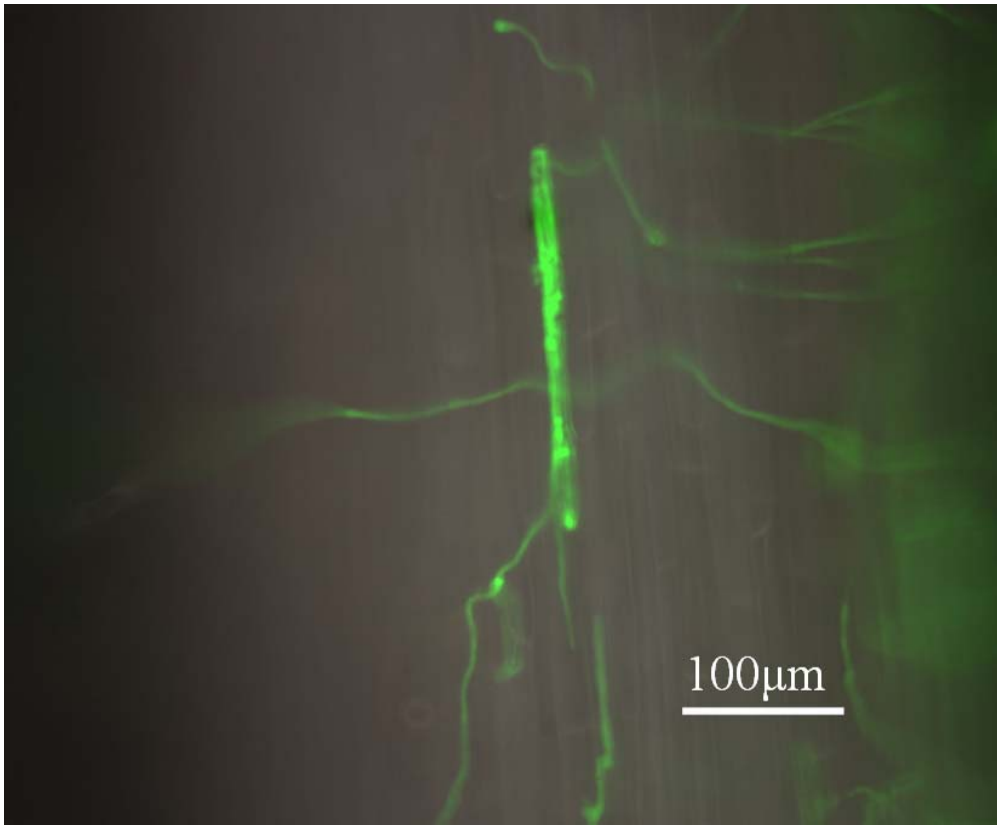

Figure A-16. ***Cynodon transvaalensis*** **root colonized by GFP expressing isolate of** ***Ophiosphaerella herpotricha*** **2 DPI (17 °C, 12 h light).**

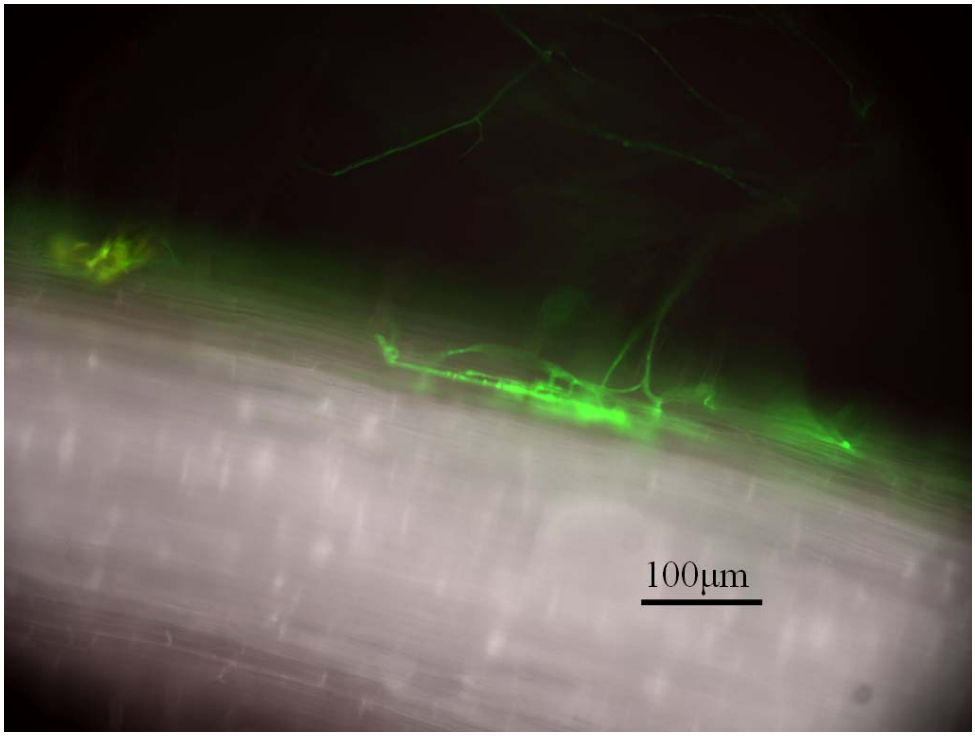

Figure A-17. *Cynodon transvaalensis* root colonized by GFP expressing isolate of *Ophiosphaerella herpotricha* at 4 DPI (17 °C, 12 h light).

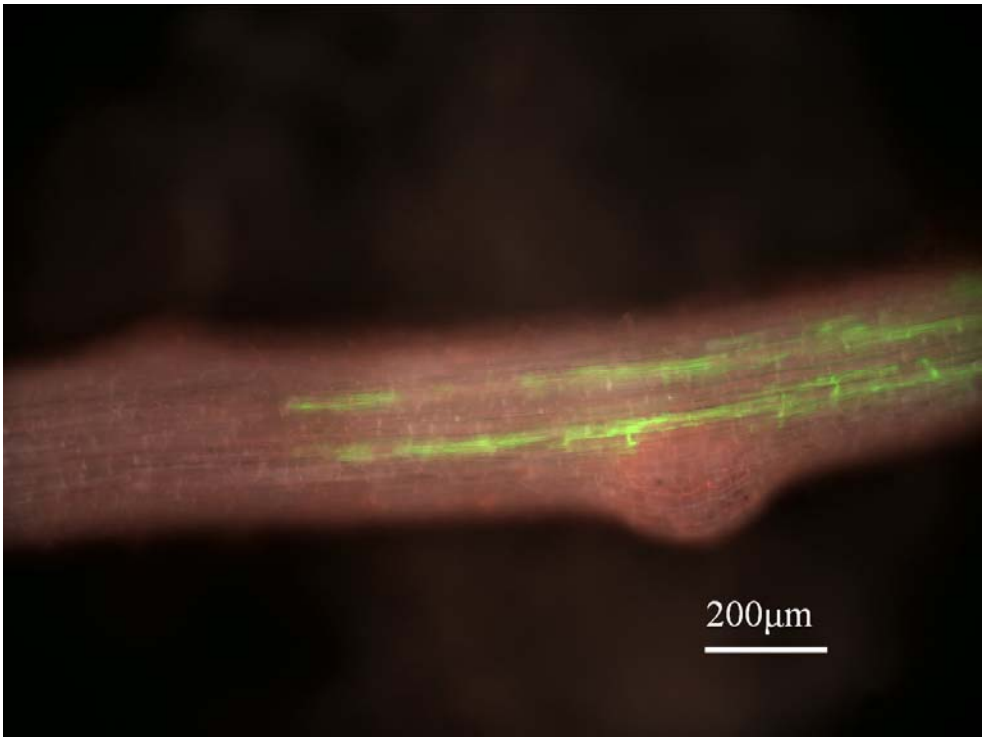

Figure A-18. ***Cynodon transvaalensis*** **root colonized by GFP expressing isolate of** ***Ophiosphaerella herpotricha*** **at 8 DPI (17°C, 12 h light).**

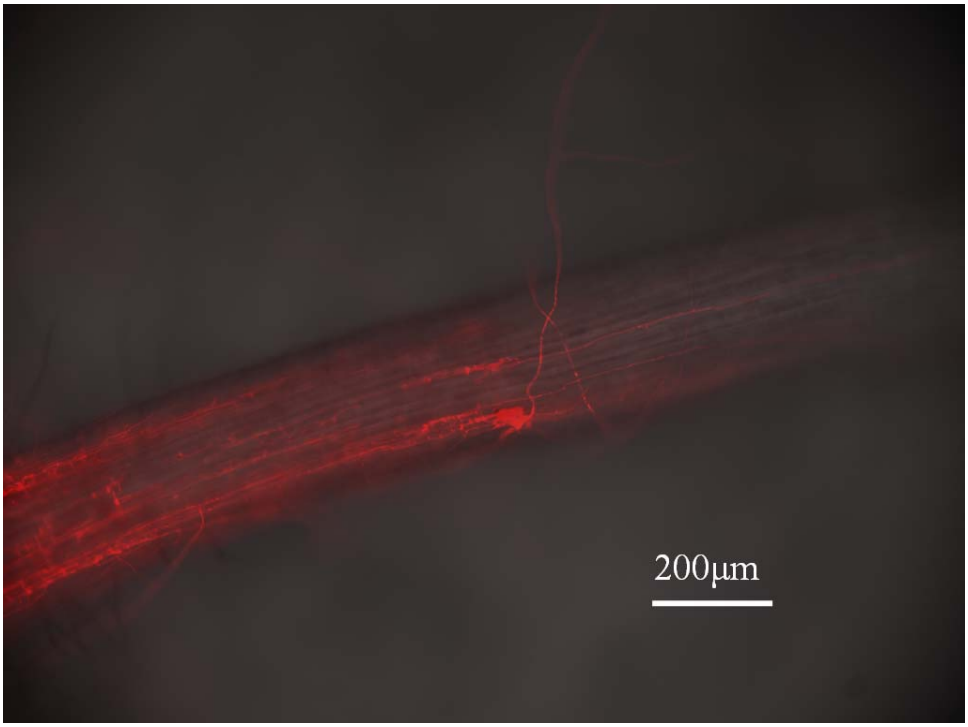

Figure A-19. ***Cynodon transvaalensis*** **root colonized by tdTom expressing isolate of** ***Ophiosphaerella herpotricha*** **at 8 DPI (17°C, 12 h light).**

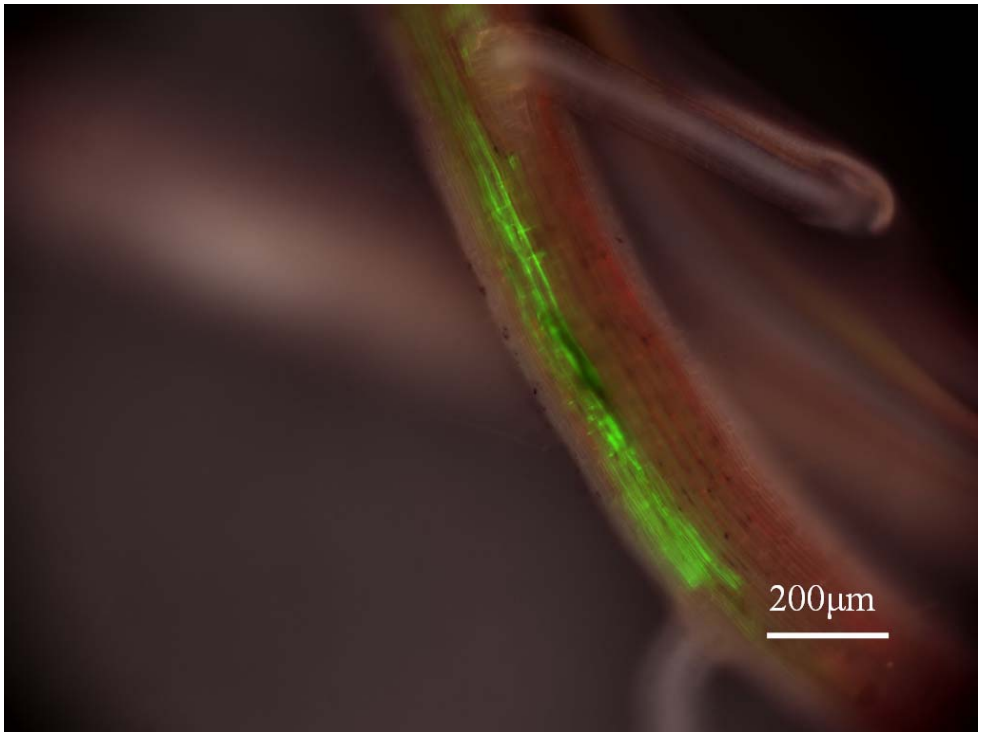

Figure A-20. ***Cynodon transvaalensis*** **root colonized by GFP expressing isolate of** ***Ophiosphaerella herpotricha*** **at 10 DPI (17°C, 12 h light).**

VITA

Oliver Cahiga Caasi

Candidate for the Degree of

Master of Science

Thesis: INFECTION AND COLONIZATION OF TURF-TYPE BERMUDAGRASS BY *OPHIOSPHAERELLA HERPOTRICHA* EXPRESSING GREEN (GFP) OR RED (TDTOM) FLUORESCENT PROTEINS

Major Field: Entomology and Plant Pathology

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Professional Memberships: American Phytopathological Society, Southern Division of the American Phytopathological Society, Gamma Sigma Delta Honor Society of Agriculture

Name: Oliver C. Caasi Date of Degree: July, 2008

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: INFECTION AND COLONIZATION OF TURF-TYPE BERMUDAGRASS BY *OPHIOSPHAERELLA HERPOTRICHA* EXPRESSING GREEN (GFP) OR RED (TDTOM) FLUORESCENT PROTEINS

Pages in Study: 104 Candidate for the Degree of Master of Science

Major Field: Entomology and Plant Pathology

Objective and Method of Study: Turf-type bermudagrasses were used to study infection and colonization by isolates of *O. herpotricha* expressing green (GFP) and red (tdTom) fluorescent proteins. The presence and location of *O. herpotricha* and necrosis were observed using epi-fluorescence microscopy.

Findings and Conclusions: Overall, *C. transvaalensis* colonization by *O. herpotricha* was less dense, less aggregated, and less necrotic than Tifway and Midlawn. Tifway was more susceptible in terms of visible macroscopic root surface necrosis and extent of epidermal colonization. Numerous stroma or aggregates of hyphae were observed on Tifway and Midlawn root surfaces. Stele colonization was observed in *C. transvaalensis* roots but not in Tifway and Midlawn. Visible necrotic lesions were also observed on Tifway and Midlawn stolons. Mat-like structures were also observed at the point of attachment. No cortical colonization was observed on Tifway, Midlawn, and the *C. transvaalensis* at 28 days post-inoculation when stolons were inoculated at internodes without wound.

ADVISER'S APPROVAL: Dr. Nathan R. Walker