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Evaluation of Ultradwarf Bermudagrass Cultural Management Practices and Identification, Characterization, and Pathogenicity of Ectotrophic Root-Infecting Fungi Associated with Summer Decline of Ultradwarf Bermudagrass Putting Greens

Phillip Lavelle Vines

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By

Phillip Lavelle Vines

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This research addressed the effects of cultural management practices, cultivar selection, and applications of seasonal fungicides on ultradwarf bermudagrass health and playability and occurrence of foliar diseases. Additionally, novel ectotrophic root-infecting fungi were isolated from ultradwarf bermudagrass roots exhibiting symptoms of summer decline, identified via multilocus phylogenetic analyses, and characterized by morphological assessments and pathogenicity evaluations.

DEDICATION

This thesis is dedicated to my parents, James and Tammie, and to my beautiful wife, Elizabeth. Words could never fully express my feelings for these individuals. In addition to providing unending love and support, my parents instilled a genuine sense of hard work and dedication in me that enabled me to succeed in the completion of this thesis. I love them dearly, forever and always.

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NOMENCLATURE

ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
BI	Bayesian Inference
BRD	Ball Roll Distance
C	Centigrade
Cv	Cultivar
CBS	Centraalbureau voor Schimmelcultures
Cc	*Candidacolonium cynodontis*
DF	Degrees of Freedom
DNA	Deoxyribonucleic Acid
DRSA	Diseased Root Surface Area
DSC	Dollar Spot Counts
ERI	Ectotrophic Root-Infecting
F	Fungicide Program
FFO	Frequency of Fungal Occurrence
FS	Fungal Species
G	Gamma distribution
GC	Growth Chamber
gDNA	Genomic Deoxyribonucleic Acid

Ggg	*Gaeumannomyces graminis* var. *graminis*
Gp	*Gaeumannomyces paulograminis*
GRW	Gravimetric Root Weight
GSRFW	Gravimetric Stolon, Root, and Foliage Weight
GTR	General Time Reversible
HKY	Hasegawa-Kishino-Yano
HRSA	Healthy Root Surface Area
I	Invariable rates among sites
ITS	Internal Transcribed Spacer
JC	Jukes-Cantor
K2	Kimura 2-Parameter
LSS	Leaf Spot Severity
LSU	Large Subunit
MCM7	Minichromosome Maintenance Complex Component 7
Mc	*Magnaporthiopsis cynodontis*
Mh	*Magnaporthiopsis hawaiiensis*
Mi	*Magnaporthiopsis incrustans*
ML	Maximum Likelihood
MSU	Mississippi State University
Mt	*Magnaporthiopsis taurocanis*
NDVI	Normalized Difference Vegetation Index
NS	Not Significant
P	Cultural Practice

PAR	Photosynthetically Active Radiation
Pc	*Pseudophialophora cynodontis*
PCR	Polymerase Chain Reaction
PD	Percent Disease
PDA	Potato Dextrose Agar
RPBI	RNA Polymerase II Gene
RRFRC	Rodney R. Foil Research Center
rRNA	Ribosomal Ribonucleic Acid
SC	Scarify
SF	Surface Firmness
SL	Slice
SP	Spike
SSU	Small Subunit
T	Time
TCM	Turf Color Meter
TEF1	Translation Elongation Factor 1-Alpha
TNN	Total Number of Nodes
TNRPN	Total Number of Root-producing Nodes
TQ	Turf Quality
T92	Tamura 3-Parameter
TRL	Total Root Length
TN93	Tamura-Nei
U.S.	United States

USA	United States of America
USGA	United States Golf Association
UTC	Untreated Control
VM	Vertical Mow
VM + SC	Vertical Mow plus Scarify
VM + SL	Vertical Mow plus Slice
VM + SP	Vertical Mow plus Spike
Y	Year

CHAPTER I

LITERATURE REVIEW

History of bermudagrass

Bermudagrass (*Cynodon* L.C. Rich) has undertaken numerous characteristic and application changes since its introduction to the United States from Africa in 1751 (Hanson, 1972). Bermudagrass was transported to the U.S. via contaminated hay that was used as bedding on ships traveling to America (Kopec, 2003). Seed were deposited onto soils of the eastern U.S., reproduced, and spread throughout other portions of America. Farmers quickly noticed forage potential for bermudagrass, which led to greater distribution across the southern U.S. By the early 20th century, bermudagrass was sought for use in home lawns and golf courses and was eventually adopted as an alternative to sand golf greens (Kopec, 2003).

The earliest bermudagrass putting greens of the 20th century were predominated by common seeded bermudagrasses (*Cynodon dactylon* (L.) Pers.) (Beard and Sifers, 1996). Golf course superintendents noticed areas on their seeded greens that did not grow as tall as the rest of the putting green. A major breakthrough in bermudagrass putting green development occurred in the 1940s when Dr. Glenn W. Burton of the Georgia Coastal Plain Experiment Station collected, increased, planted, and evaluated phenotypic mutants from putting greens throughout the southern U.S. (Burton, 1991).

As a result of Dr. Burton's work, 'Tiflawn' bermudagrass was released in 1952 (Burton, 1991; Hanna and Anderson, 2008). Tiflawn was a great option for home lawn applications, but was too coarse to use for golf greens; therefore, it was crossed with a fine-leafed bermudagrass to produce 'Tiffine', which was released in 1953 (Hein, 1953; Burton, 1991). Tiffine had finer, softer leaves, was more suitable for golf green use, and was sterile. Three years later, in 1956, 'Tifgreen' was released (Burton, 1991).

Tifgreen was the resultant product of the cross between a common bermudagrass from a golf course country club in North Carolina and an Egyptian *Cynodon transvaalensis* Burtt-Davy (Burton, 1991; Hanna and Anderson, 2008). Tifgreen had finer, softer, dark green leaves, minimal seedheads, and could withstand daily mowing heights of 4.7 mm (Burton, 1991; Kopec, 2003; Hanna and Anderson, 2008). In the early 1960s, off-types in Tifgreen putting greens arose and appeared to possess more desirable qualities (Burton, 1991; Kopec, 2003). The off-types were first tested in 1962, leading to the release of 'Tifdwarf' in 1965. Shortly after the release of Tifdwarf, the new release replaced Tifgreen (Burton, 1991; Hanna and Anderson, 2008).

Tifdwarf could endure daily mowing heights near 4 mm, which led to extensive use on putting greens throughout parts of the southern U.S. (Hanna and Anderson, 2008). As time progressed, off-types of this grass were observed in many putting greens (Moncrief, 1975). A large number of today's ultradwarf bermudagrasses are derivatives from vegetative mutants within Tifdwarf golf greens (Kopec, 2003; Hanna and Anderson, 2008). Popular ultradwarfs utilized on golf courses today include 'Champion', 'MiniVerde' (Fig. 1.1), and 'Tifeagle'. Each of these ultradwarfs can be routinely

mowed at 3.2 mm or less (Rowland, 2011). Historical events in bermudagrass development, from introduction to current usage, are summarized in Fig. 1.2.

Figure 1.1 MiniVerde ultradwarf bermudagrass at number 1 green of The Player's Club at Sawgrass in Ponte Vedra Beach, Florida.

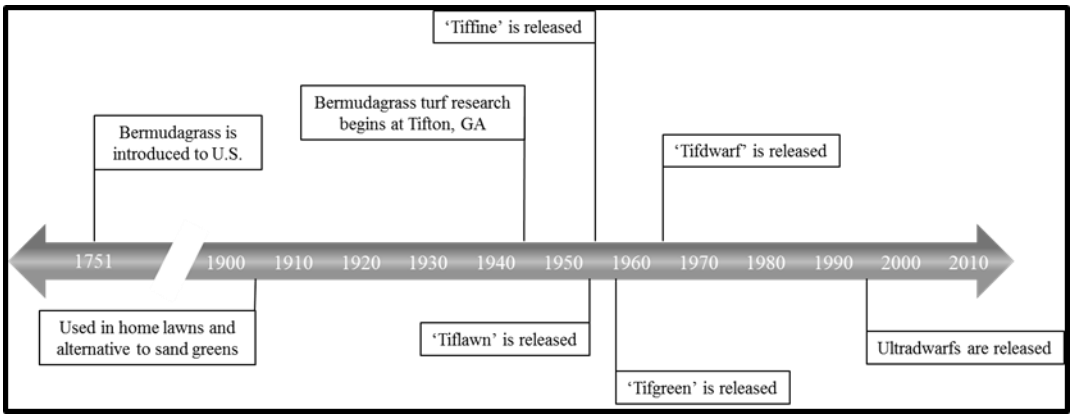

Figure 1.2 Chronology of historical events in the development of ultradwarf bermudagrasses currently used as golf greens.

Cultural management of ultradwarf bermudagrass putting greens

Ultradwarf bermudagrass putting greens display traits of fine leaf blades, short internodes, high shoot densities, rapid recuperative potentials, and reduced growing heights (Fig. 1.3) (Beard and Sifers, 1996; Guertal and White, 1998; Guertal et al., 2001; White et al., 2004). Although each of these traits are desirable for putting greens, they contribute to an increased accumulation of dead and decaying plant material, known as thatch, located just above the soil line (Decker, 1974; Turgeon, 2005; McCarty et al., 2007; Fontanier et al., 2011). Thatch, when maintained at appropriate levels, provides a cushioning region that protects living plant material from detrimental stresses posed by harsh impacts such as foot and vehicular traffic (Smith, 1979; Bevard, 2005). However, negative aspects arise when excessive amounts of thatch accumulate (Turgeon, 2005; McCarty et al., 2007).

Figure 1.3 Ultradwarf bermudagrass maintained at 3.2 mm height of cut at the Rodney R. Foil Plant Science Research Center in Starkville, MS.

When thatch material becomes intermingled with soil particles, a mat layer is formed (Decker, 1974; Turgeon, 2005; McCarty et al., 2007; Fontanier et al., 2011). Thatch and mat adversely affect normal functions of bermudagrass putting greens (Murray and Juska, 1977; Bevard, 2005; McCarty et al., 2007). These factors decrease water and oxygen infiltration (Cornman, 1952; Musser, 1960; Murray and Juska, 1977; Bevard, 2005), increase insect and disease pressure (Cornman, 1952; Musser, 1960; Murray and Juska, 1977; Bevard, 2005; Turgeon, 2005), reduce efficacy of pesticide applications (Musser, 1960), and generate a greater occurrence of ball roll inconsistences

on putting greens (Vermeulen and Hartwiger, 2005). Management of thatch and mat has been emphasized in numerous research efforts to date.

Core cultivation

Core cultivation (Figs. 1.4 and 1.5), sometimes referred to as aerification, core aeration, or coring, is a management practice in which cores, containing plant and soil materials, are removed from the soil profile of putting greens. This practice is effective in reducing soil organic matter to desired levels (Bevard, 2005; Turgeon, 2005; McCarty et al., 2007). Voids that remain on putting green surfaces are commonly replaced with sand, and turfgrass plants fill in these areas. Benefits of core cultivation include increased water infiltration and gas exchange (Canaway et al., 1986; Murphy and Rieke, 1994; Bunnell et al., 2001; McCarty et al., 2007), reduced levels of thatch and mat (Murray and Juska, 1977; Smith, 1979; Eggens, 1980; Murphy and Rieke, 1994), and improved root health; however, there are major disadvantages to this procedure. A few weeks are required for recuperation from core aeration, and both playability and aesthetics of putting surface are immediately reduced (Fontanier et al., 2011). These shortcomings have led to an increased demand for less destructive thatch management alternatives.

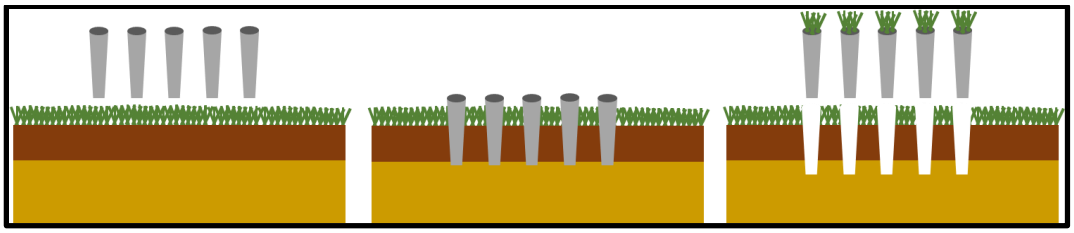

Figure 1.4 Schematic illustration of hollow-tine aerification.

Figure 1.5 Hollow-tine core aerification of research plots at the Rodney R. Foil Plant Science Research Center in Starkville, MS.

Venting cultivation

A less disruptive alternative to core removal is venting cultivation, whereby slits or small holes are created in the upper portion of the soil profile (Fig. 1.6). Methods of venting include needle-tine aeration, spiking, slicing, and pressurized water injection (Green et al., 2001; Turgeon, 2005; Fontanier et al., 2011). Each of these practices is effective in creating channels that increase water and gaseous movement, but do not directly remove thatch and organic material from the soil profile (Murphy and Rieke, 1994; Green et al., 2001; Fontanier et al., 2011). However, increased gas and water movement enhances microbial populations, leading to improved degradation of thatch material (Cornman, 1952; Turgeon, 2005).

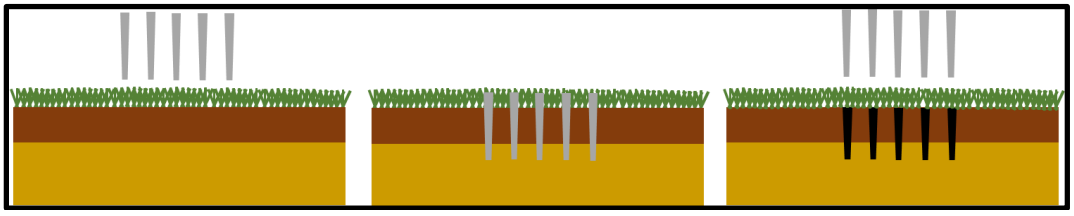

Figure 1.6 Schematic illustration of venting aeration conducted with needle tines.

Vertical mowing and sand topdressing

Additional management strategies for thatch control include vertical mowing and sand topdressing (Bevard, 2005; Turgeon, 2005; McCarty et al., 2007). Vertical mowing, also known as dethatching or power raking, is a practice that utilizes blades, which rotate in a perpendicular plane to that of the putting green surface (Turgeon, 2005; McCarty et al., 2007). As penetration depth of vertical mowing blades increases, more thatch is disrupted, surfaced, and removed (Turgeon, 2005). Sand topdressing of ultradwarf

bermudagrass putting greens is a technique similar to venting aeration in that it does not directly result in a decrease of thatch material, but can increase microbial degradation of thatch material (Turgeon, 2005).

Ectotrophic root-infecting fungi and associated diseases

The soil-borne, root-infecting group of fungi, commonly referred to as ectotrophic root-infecting (ERI) fungi, encompasses several genera that affect various types of plants including grain crops and grasses (Clarke and Gould, 1993). These fungi colonize plant roots via darkly pigmented, ectotrophic, robust runner hyphae (Figs. 1.7 and 1.8) (Clarke and Gould, 1993; Tredway, 2006). ERI fungi, when active on turfgrass plants, cause circular or patch-like dieback within turfgrass stands and have been considered the cause of some of the most destructive turfgrass diseases in the U.S. (Landschoot and Jackson, 1990; Wetzel et al., 1996). Symptoms of this group of fungi are generally observed in recurring locations at annual or sporadic periods (Smiley et al., 2005).

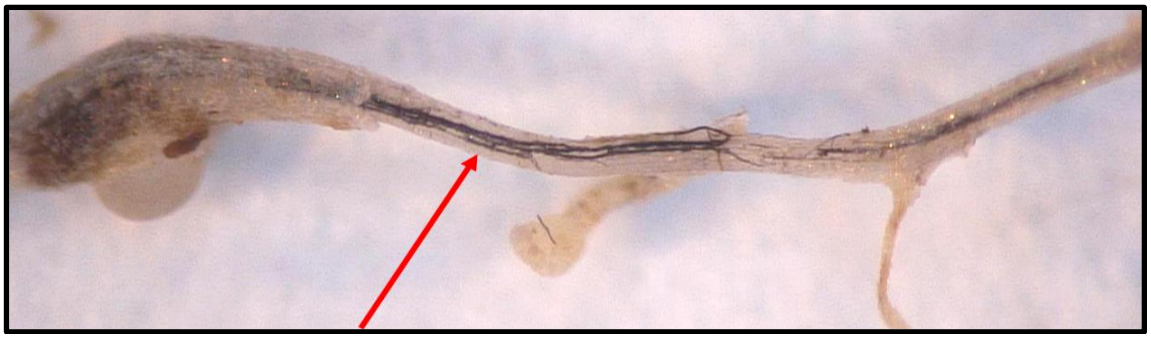

Figure 1.7 Dark, runner hyphae on exterior of ultradwarf bermudagrass root.

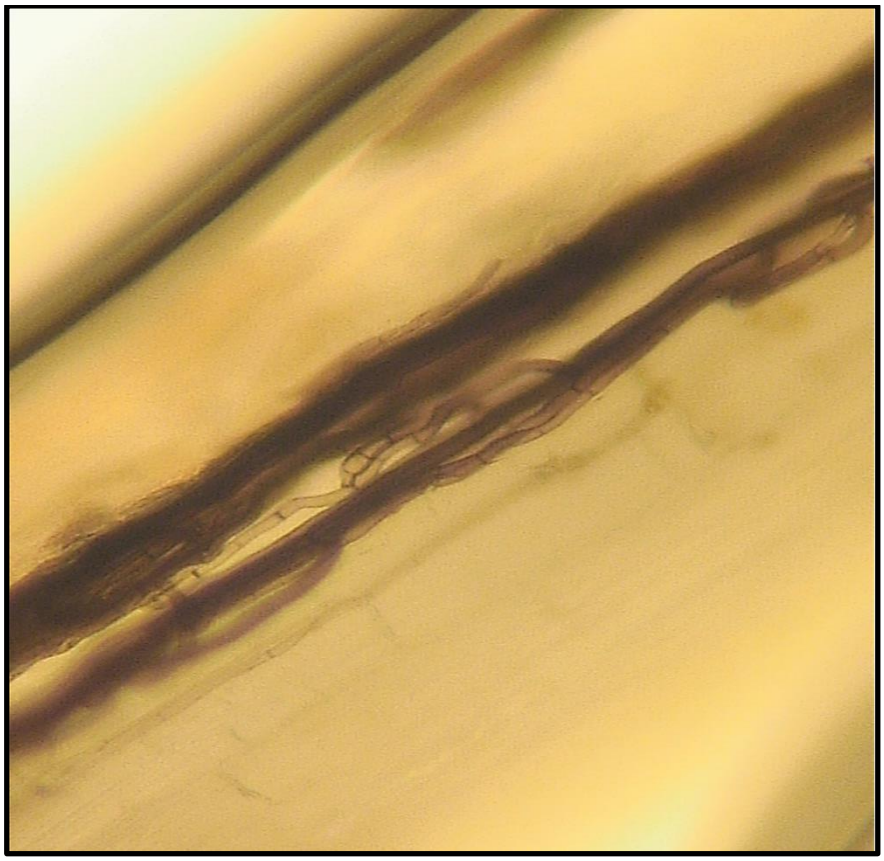

Figure 1.8 Magnified image of dark, runner hyphae on ultradwarf bermudagrass root.

Turfgrass diseases of this nature have occurred for more than 60 years (Smiley et al., 2005). *Gaeumannomyces graminis* (Sacc.) Arx and D. Olivier var. *avenae* (E. M. Turner) Dennis, the causal agent of take-all patch, was the sole member of this group of fungi prior to 1984 (Clarke and Gould, 1993). Since that time, it has become evident that other well-defined patch-like diseases are caused by *Gaeumannomyces*-type fungi (Smiley et al., 2005).

Take-all patch

As stated, *G. graminis* var. *avenae* (≡ *Ophiobolus graminis* (Sacc.) Sacc. var. *avenae* E. M. Turner), the causal agent of take-all patch, is a destructive pathogen of bentgrass (*Agrostis* L.) turf (Dernoeden, 1987; Smiley, 2005). This organism also affects fescues (*Festuca* L.) and bluegrasses (*Poa* L.); however, the threat is less severe than with bentgrass (Smiley et al., 2005). Symptoms of take-all patch arise during late spring or early summer as small, reddish brown or light brown patches, and linger into the latter summer months if cool, moist environmental conditions are present (Smiley et al., 2005). The fungus survives winter as dormant mycelium in previously infected host plant material and infects stolons, roots, and rhizomes during spring and fall (Smiley et al., 2005).

Bermudagrass decline

Gaeumannomyces graminis (Sacc.) Arx and D. Olivier var. *graminis*, the causal agent of bermudagrass decline, survives adverse environmental conditions as mycelium, colonizing roots, stolons, and rhizomes of bermudagrass plants (Smiley et al., 2005). Symptoms of bermudagrass decline appear during warm to hot periods of the summer when humidity levels are high (Elliott, 1991; Smiley et al., 2005). After colonization and infection by the fungus, lower leaves of bermudagrass plants become chlorotic and roots are shortened and discolored (Elliott and Landschoot, 1991). Field symptomatology includes irregularly shaped chlorotic patches, which vary in diameter from 0.2 to 1 m (Elliott and Landschoot, 1991; Smiley et al., 2005).

G. graminis var. *graminis* causes a similar disease, take-all root rot, in other warm-season turfgrass species. Take-all root rot affects St. Augustinegrass

(*Stenotaphrum secundatum* (Walt.) Kuntze), seashore paspalum (*Paspalum vaginatum* Sw.), centipedegrass (*Eremocholoa ophiuroides* (Munro) Hack.), and zoysiagrass (*Zoysia japonica* Steud.). Symptomatology and disease development are similar to bermudagrass decline (Smiley et al., 2005). Collectively, bermudagrass decline and take-all root rot are recognized as root decline of warm-season turfgrasses (Smiley et al., 2005).

Summer patch

Summer patch, caused by *Magnaporthiopsis poae* (Landschoot and Jackson) J. Luo and N. Zhang (≡ *Magnaporthe poae* Landschoot and Jackson), is a disease that primarily affects Kentucky bluegrass (*Poa pratensis* L.) and fine-leaf fescues such as strong creeping red fescue (*Festuca rubra* subsp. *rubra*), slender creeping red fescue (*F. rubra* var. *littoralis*) hard fescue (*F. brevipila*), Chewings fescue (*F. rubra* subsp. *fallax*), and sheep fescue (*F. ovina*) (Clarke and Gould, 1993; Landschoot et al., 1993; Smiley et al, 2005). *M. poae* has been isolated from perennial ryegrass (*Lolium perenne* L.) and bentgrass, but is not considered a primary threat to those species (Smiley et al., 2005). However, creeping bentgrass managed at putting green height can be greatly affected (Landschoot et al., 1993; Smiley et al., 2005).

M. poae overwinters as dormant mycelium in previously colonized plant material (Smiley et al., 2005). Symptoms begin to appear on Kentucky bluegrass in early summer months as small, circular patches of slow growing turf, which expand to approximately 30 cm, but may exceed 1 m in diameter (Smith et al., 1989; Smiley et al., 2005). Patches of affected turf fade from grayish green to reddish brown, and ultimately become straw-colored or tan (Smiley et al., 2005). Colonization of plant material occurs between temperatures of 20 to 35 C under controlled conditions with optimum temperatures of 28

to 30 C. (Kackley et al., 1990; Smiley et al., 2005). Field symptoms are most prevalent in the presence of heavy rainfall followed by high temperatures (Sreedhar et al., 1999; Smiley et al., 2005).

Dead spot

Ophiosphaerella agrostis P. H. Dernoeden, M. Camara, N. O'Neill, van Berkum, and M. Palm, the causal agent of dead spot, poses threats to bentgrasses and bermudagrasses (Dernoeden et al., 1999; Krausz et al., 2001; Smiley et al., 2005). Symptoms on golf greens begin as small (1 cm diameter), reddish brown discolorations of turf and progress into larger (9 cm diameter) patches (Dernoeden et al., 1999; Kaminski, 2004; Smiley et al., 2005). Matured disease symptoms include patches with reddish, brown borders surrounding tan or straw-colored centers and discolored, necrotic root systems (Dernoeden et al., 1999; Kaminski, 2004; Smiley et al., 2005). In bentgrass, the disease occurs from May to December with most prevalent symptoms arising during hot and dry summer months (Kaminski, 2004; Smiley et al., 2005; Kaminski and Dernoeden, 2006). On bermudagrass, dead spot is observed during spring green-up from March to April. In May, as temperatures increase, bermudagrass plants begin to thrive and rapidly recuperate from disease symptoms (Kaminski and Dernoeden, 2005; Smiley et al., 2005). The fungus overwinters as pseudothecia in crowns, roots, and stolons of turfgrass plants (Kaminski, 2004; Smiley et al., 2005; Kaminski and Dernoeden, 2006). As temperatures approach or surpass 20 C for a sustained period, the fungus becomes active and begins to invade tissues of turfgrass plants (Kaminski, 2004; Smiley et al., 2005).

Necrotic ring spot

Necrotic ring spot, caused by *O. korrae* (J. Walker and A. M. Sm.) Shoemaker and Babcock (≡ *Leptosphaeria korrae* J. Walker and A. M. Sm. and *Ophiobolus herpotricha* (Fr.:Fr.) J. Walker), occurs during periods of cool, wet weather on numerous cool-season turfgrasses (Clarke and Gould, 1993; Smiley et al., 2005). This disease can affect annual bluegrass (*Poa annua* L.), red fescue (*Festuca rubra* L.), and roughstalk bluegrass (*Poa trivialis* L.); however, it is considered most destructive to Kentucky bluegrass (Clarke and Gould, 1993; Smiley et al., 2005). Disease symptoms first appear as small (5 to 10 cm diameter) yellowish green areas of turf, which progress into larger (30 cm), reddish brown patches (Smiley et al., 2005). Advanced symptoms include straw-colored patches, which are normally less than 30 cm in diameter, but occasionally exceed 60 to 90 cm in diameter (Smiley et al., 2005). Additionally, severe rot of root systems is noted (Smiley et al., 2005) Necrotic ring spot is exacerbated during hot, dry periods when turfgrass plants become stressed (Smiley et al., 2005).

Spring dead spot

O. herpotricha (Fr.:Fr.) J. Walker, *O. korrae* (J. Walker and A. M. Sm.) Shoemaker and Babcock (≡ *Leptosphaeria korrae* J. Walker and A. M. Sm.), and *O. narmari* (J. Walker and A. M. Sm.) Wetzel, Hulbuer, and Tisserat (≡ *Leptosphaeria narmari* J. Walker and A. M. Sm.) are three fungal species known to incite spring dead spot disease of bermudagrass and buffalograss (*Buchloe dactyloides* (Nutt.) Columbus) (Smiley et al., 2005; Perry, 2008). Spring dead spot is considered the most destructive disease of bermudagrass in North America (Smiley et al., 2005). Although each fungal species incites spring dead spot, the three species occur in distinct regions of the U.S.

(Walker and Smith, 1972; Tisserat et al., 1989; Dernoeden et al., 1995; Wetzel et al., 1999; Iriarte et al., 2004). *O. korrae* is most commonly associated with spring dead spot in the eastern U.S. and southern California (Endo and Krausman, 1985; Dernoeden et al., 1995; Iriarte et al., 2004), while *O. herpotricha* is generally located in the Great Plains region of the western U.S. (Tisserat et al., 1989; Wetzel et al., 1999). *O. narmari* is observed throughout the U.S. and in Australia (Walker and Smith, 1972; Tisserat et al., 1994; Smiley et al., 2005).

Growth of spring dead spot fungi is most rapid in winter months when soil temperatures are relatively cool, 10 to 25 C, and soil is moist (Crahay et al., 1988; Kackley et al., 1990; Smiley et al., 2005; Perry et al., 2010). This is not the case for roots of bermudagrass plants, which thrive at soil temperatures of 35 C and regress in growth at temperatures of 15 C (Smiley et al., 2005). This presents an advantageous setting for spring dead spot fungi to attack roots of bermudagrass (Tisserat et al., 1994; Smiley et al., 2005). Field symptoms appear as sunken, necrotic, bleached patches of turf that occur during the transitional period from winter dormancy to spring green-up (Smiley et al. 2005; Perry, 2008). Symptoms regularly recur in the same areas for consecutive years; however, size and shape of desiccated turf may be variable (Perry, 2008). The fungi produce dark brown, septate mycelial mats on roots and stolons of infected plants, and ascocarps are occasionally observed on dead tissues (Smiley et al. 2005).

Identification of ERI fungi

Accurate identification of ERI fungi via traditional diagnostic methods is generally very difficult and time-consuming (Bryan et al., 1995; Wetzel et al., 1996; Rachdawong et al., 2002). With the exception of *G. graminis* var. *graminis*, which is

readily identified by deeply lobed hyphopodia, ERI fungi do not commonly produce distinguishable identification features, inhibiting precise, timely identification (Wetzel et al., 1996). Fungi of this group must be isolated from infected plant material, and ascocarp formation must be induced for precise morphological identification (Wetzel et al., 1996). Production of ascocarps is often unsuccessful because isolates may be of incompatible mating types or attenuated biotypes (Wetzel et al., 1996). If appropriate biotypes and mating types are acquired, a period of four to ten weeks is required for production of identification structures (Dernoeden and O'Neill, 1983; Crahay et al., 1988; Landschoot and Jackson, 1989a; Landschoot and Jackson, 1989b). Because of rigorous and rather demanding morphological identification measures, numerous molecular-based identification strategies have been studied for identification of ERI fungi (Huff et al., 1994; Wetzel et al., 1996).

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CHAPTER II

EFFECTS OF CULTURAL MANAGEMENT PRACTICE, CULTIVAR SELECTION, AND SEASONAL FUNGICIDE PROGRAM ON PLANT HEALTH AND PLAYABILITY OF ULTRADWARF BERMUDAGRASS PUTTING GREENS

Abstract

Golf course putting greens in the southern United States are commonly grassed with an ultradwarf bermudagrass cultivar. In this setting, ultradwarfs exhibit high shoot densities, fine leaf textures, rapid recuperative abilities, and prostrate growing habits, enabling them to endure decreased mowing heights. These desirable characteristics frequently result in excessive accumulations of organic matter that can be unfavorable for plant health and playability. To remediate inflated amounts of thatch and organic matter, vertical mowing is commonly conducted in a frequently recurring manner. Vertical mowing is effective at decreasing thatch and organic matter; however, plant health and playability is often sacrificed. This research evaluated the influences of less-aggressive and less frequently applied slicing, spiking, and scarifying treatments on ultradwarf bermudagrass health and playability. The study was conducted as a randomized complete block design with a split-plot constraint with three replications of 'Champion' and 'MiniVerde' ultradwarf bermudagrass. Cultural management practices were main-plot factors and levels of fungicide were split-plot factors. Plant health was assessed as

turf quality and normalized difference vegetation index, and playability evaluated as ball roll distance and surface firmness. The best treatments were incorporations of less-aggressive slice, spike, and scarify practices into vertical mow regimes once each month. These treatments improved the turf characteristics associated with playability without sacrificing plant health.

Introduction

Ultradwarf bermudagrass cultivars have short internodes, increased shoot production, fine-textured leaves, and prostrate growing habits, permitting reduced heights of cut (Beard and Sifers, 1996; Guertal and White, 1998; Guertal et al., 2001; White et al., 2004). These traits are desirable for golf course putting greens, but contribute to an increased accumulation of thatch, which is dead and decaying organic material located directly above the soil line (Decker, 1974; Turgeon, 2005; McCarty et al., 2007; Fontanier et al., 2011). Thatch is primarily composed of stolons, rhizomes, maturated leaf sheaths and blades, and intermittently sloughed roots (Engel, 1954; Roberts and Bredakis, 1960). Thatch depths between 0.6 and 1.3 cm deliver a cushion for living plant material, defending against damages posed by harsh impacts such as foot and vehicular traffic (Smith, 1979; White and Dickens, 1985; Bevard, 2005). As thatch levels exceed 1.3 cm, disadvantageous characteristics develop (White and Dickens, 1984; Turgeon, 2005; McCarty et al., 2007).

A mat layer is formed as thatch becomes intermixed with soil particles (Decker, 1974; Turgeon, 2005; McCarty et al., 2007; Fontanier et al., 2011). Thatch and mat are known to decrease oxygen and water infiltration, increase disease and insect pressure, generate a greater occurrence of ball roll inconsistences, and reduce efficacy of pesticide

applications (Cornman, 1952; Musser, 1960; Murray and Juska, 1977; Bevard, 2005; Turgeon, 2005; Vermeulen and Hartwiger, 2005; McCarty et al., 2007). Common management practices for thatch control of ultradwarf bermudagrass cultivars include frequent vertical mow (VM) and sand topdressing applications (Bevard, 2005; Turgeon, 2005; McCarty et al., 2007; Rowland, 2011; Lowe, 2013).

VM is a practice in which blades rotate in a perpendicular plane to that of the putting green surface (Turgeon, 2005; McCarty et al., 2007). As penetration depth of VM blades increases, more thatch is disrupted, surfaced, and removed (Turgeon, 2005). Plant injury is associated with VM practices; however, little research has been conducted to compare injurious effects of VM to less-aggressive, alternative cultural management practices such as slicing, spiking, and scarifying (McCarty et al., 2007; Uddin et al., 2008).

Slicing is a method of turf cultivation conducted by insertion of vertically rotating, V-shaped blades through the turf canopy, into the soil (Turgeon, 1997; Beard and Beard, 2005). Spiking is a process similar to slicing in which flat, pointed blades or solid tines penetrate turf and soil surface. Penetration depth is limited to the length of spikes, usually less than 25 mm (Turgeon, 1997; Beard and Beard, 2005). Unlike VM, less-aggressive practices such as slicing and spiking do not remove organic matter; however, slits are made, increasing water and gaseous exchange and enhancing root and shoot growth (Turgeon, 1997).

In practical terms, scarification cultivation practices are synonymous with VM practices in that vertical tines are employed to remove thatch and green vegetation from

turf (Beard and Beard, 2005). However, scarification devices are available that provide quality playability conditions immediately after application.

The purpose of this study was to examine the influences of less readily employed cultural management practices, in combination with cultivar selection and chemical fungicide applications, on health and playability characteristics of ultradwarf bermudagrass putting greens.

Materials and methods

Background management of research area

A two-year study was conducted in Starkville, MS during 2012 and 2013 on four-year-old ultradwarf bermudagrass managed at golf course standards (Fig. A.1). The research green consisted of a 90:10 sand:peat root zone mix with a pH of 6.5. Mowing events occurred daily at 3 to 4 mm height of cut with a Greensmaster ® Flex™ 2100 walk-behind greens mower (The Toro Company, Bloomington, MN, USA). Turf was irrigated deep and infrequently to promote vertical root growth, maintain moisture, and avoid drought stress.

Annual fertilizer applications supplied 293 kg N ha^{-1}, 98 kg P ha^{-1}, and 342 kg K ha^{-1}. Primo MAXX® (trinexapac-ethyl, Syngenta, Greensboro, NC, USA) was applied at 0.026 kg a.i. ha^{-1} $week^{-1}$ during June, July, and August. Cores, 7.6 to 10.2 cm long, were removed with 1 cm wide hollow tines spaced 3.8 cm apart on 18 Jun 2012 and 16 Jul 2013. Aerification events were conducted with a Procore® 648 (The Toro Company, Bloomington, MN, USA) and followed by sand topdressing to fill surface voids.

Experimental design and analysis

A randomized complete block design with a split-plot constraint with three replications of each cultivar was used for the study (Fig. A.2). Ultradwarf cultivars used were 'Champion' and 'MiniVerde'. Main-plot (2.1 × 2.4 m) factors were cultural practices and split-plot (2.1 × 1.2 m) factors were levels of fungicide. The MIXED procedure in SAS v. 9.3 (SAS Institute Inc., Cary, NC, USA) was employed for statistical analyses. Multiple comparisons were computed with least squares means for a given effect when the F-value was ≥ 4.0 and the F-ratio was significant at the 0.05 level. The F-value of 4.0 was chosen to systematically screen statistically significant results for practical significance, since the error degrees of freedom was large for many of the F-tests. Numerous high-order interactions were statistically significant, but from a practical standpoint, they were not meaningful.

Description and timing of treatment applications

There were two levels of fungicide in the study, either presence or absence of a seasonal fungicide spray regime. In 2012 and 2013, initial fungicidal applications were made in early June with Honor (pyraclostrobin + boscalid, BASF, Research Triangle Park, NC, USA) at 0.512 kg a.i. ha^{-1} and 0.342 kg a.i. ha^{-1}, respectively. An application of Interface (iprodione + trifloxystrobin, Bayer Crop Science, Research Triangle Park, NC, USA) at 3.27 kg a.i. ha^{-1} and 0.198 kg a.i. ha^{-1}, respectively, was performed in July. A single application of Heritage (azoxystrobin, Syngenta, Greensboro, NC, USA) at 0.454 kg a.i. ha^{-1} was made in August. Chipco Signature (Aluminum tris, Bayer Crop Science, Research Triangle Park, NC, USA) tank-mixed with Fore (mancozeb, Dow AgroSciences, Indianapolis, IN, USA), each at 1.83 kg a.i. ha^{-1}, was applied in

September, prior to a concluding fungicide application of Rubigan (fenarimol, Gowan Co., Yuma, AZ, USA) at 0.382 kg a.i. ha^{-1} in October.

Cultural practices were VM, vertical mow plus slice (VM + SL), vertical mow plus spike (VM + SP), vertical mow plus scarify (VM + SC), slice (SL), spike (SP), and scarify (SC) (Table A.1). Cultural practice treatments were initiated 8 Jun 2012 and 12 Jun 2013 and terminated 14 Sep 2012 and 18 Sep 2013 (Fig. A.3)

Cultural practice treatments were applied with True-Surface® Greens Care Collection inserts (Turfline Incorporated, Moscow Mills, MO, USA). VM was included in the study as a control treatment to represent a management practice readily employed by golf course superintendents. VM + SL, VM + SP, and VM + SC were combination treatments included to determine the effect of once-monthly incorporated less-aggressive, alternative cultural practices on ultradwarf bermudagrass health and playability. SL, SP, and SC treatments were applied once each month and were included to assess the effect of less frequent cultural practice applications on ultradwarf bermudagrass health and playability.

VM treatments were conducted weekly with the Vacu-Cutter™ (Fig. A.4) insert in a bidirectional, perpendicular fashion at a depth of 4 mm and blades spaced 10 mm apart. The Vacu-Cutter™ insert is composed of 45 stainless steel blades, each having 11 tungsten carbide tips. VM + SL, VM + SP, and VM + SC treatments were applied the same as VM treatments except SL, SP, and SC applications were incorporated once each month, in a unidirectional fashion with the Deep Slicer (Fig. A.5) at a depth of 19 mm with blade spacing 40 mm apart, the Greens Spiker (Fig. A.6) at a depth of 19 mm and spikes spaced 60 mm apart, and the Vacu-Scarifier™ (Fig. A.7) at a depth of 9.5 mm

with 40 mm blade spacing, respectively. SL, SP, and SC treatments were applied once each month, in a unidirectional manner as previously described.

Data collection

Turf quality (TQ) and normalized difference vegetation index (NDVI) were assessed to determine ultradwarf bermudagrass health. Playability evaluations included ball roll distance (BRD) and surface firmness (SF) (Beard, 1982; Salaiz et al., 1995). A grid was constructed from 6.4 mm thick polyvinyl chloride pipe, and consisted of 28 sections, each 0.09 m^2 (Fig. A.8). The grid was made to the same size dimensions of each sub-plot, 2.1 m x 1.2 m, and was used to guide the placement of SF and NDVI evaluation instruments. Random numbers, 1 to 28, were generated to guide in all sampling and evaluation methods.

Turf quality

Quality of turfgrass within each subplot was recorded two times each month from June through November using a visual rating scale. TQ ratings accounted for color, density, uniformity, and texture within each sub-plot. The visual rating scale ranged from 1 to 9, where 1 equated to dead turf and 9 was ideal, lush, dark green turf (Morris and Shearman, 1998).

Normalized Difference Vegetation Index

Differentiations between healthy and stressed turf have been adequately measured by spectral reflectance in previous studies (Raikes and Burpee, 1998; Bell et al., 2002; Jiang and Carrow, 2005; Kruse et al., 2006). The NDVI was developed from reflectance at visible red (R) and near-infrared (NIR) wavelength ranges and is defined as

[(NIR – R) / (NIR + R)] (Rouse et al., 1973). NDVI has been used to assess quality of turf and turf canopy characteristics in previous research on various turfgrass species (Trenholm et al., 1999; Fitz-Rodriguez and Choi, 2002, Jiang et al., 2003; Keskin et. al., 2003; Xiong et al., 2007; Sönmez et al., 2008; Jiang et al, 2009). In this study, NDVI was evaluated two times each month from June to November using a FieldScout® Turf Color Meter 500 NDVI (Spectrum Technologies, Inc., Aurora, IL, USA). The average of two NDVI samples was recorded for each sub-plot.

Ball roll distance

In 1977, the United States Golf Association (USGA) introduced the Stimpmeter to measure BRD as a means of assessing putting green speed (Beard, 1982; Oatis, 1990). A standard USGA Stimpmeter is 0.9 m in length and has a notch located 76 cm from the beveled end (Radko, 1980). The notch is designed to release a golf ball when the instrument is raised to 20 degrees above ground level (Radko, 1980). BRD of turf research plots is difficult to assess with a standard USGA Stimpmeter because of typical plot lengths; therefore, a modified Stimpmeter has been developed for use on small-plot research areas (Lodge, 1992; Gaussoin et al., 1995).

In this study, BRD was measured with a modified Stimpmeter, which measured 19 cm from ball notch to beveled end. BRD was determined for each sub-plot by rolling three golf balls in one direction, rolling them in the opposite direction, and determining the average distance traveled. BRD was assessed once monthly, June through October, in 2012 and 2013.

Surface firmness

Firmness of putting green surface was evaluated once monthly, June through November, in 2012 and 2013. SF levels were assessed via the TruFirm system (United States Golf Association, Far Hills, NJ, USA) by placing the instrument on the putting green surface, raising the plunger to its most extended level, and releasing the plunger, allowing the attached stainless steel hammer to penetrate the putting green surface. This system measures surface firmness as the depth of penetration that the hammer travels into the green. Lower penetration depths correspond to firmer putting green surfaces. The average of two SF samples was recorded for each sub-plot.

Results

Turf quality

There were 1,008 observations for TQ during the two-year study. Two interaction effects [(time × year × cultivar) and (time × year × fungicide program)] and one main effect (cultural practice) exhibited F-values ≥ 4.0 and significantly affected mean TQ (Table 2.1). These effects are discussed in following sections.

Table 2.1 ANOVA for mean turf quality (TQ) on Champion and MiniVerde ultradwarf bermudagrass in Starkville, MS from June to November in 2012 and 2013.

Source	DF	F-value	Pr > F
Rep	2	———	———
Cultivar (Cv)	1	70.02	0.014
Rep × Cv, Error 1	2	———	———
Year (Y)	1	138.9	0.0003
Y × Cv	1	0.75	0.4343
Rep(Cv × Y), Error 2	4	———	———
Cultural Practice (P)	6	22.4	<0.0001
P × Cv	6	1.44	0.2205
P × Y	6	2.4	0.0415
P × Y × Cv	6	1.13	0.3578
Rep(Y × Cv × P), Error 3	48	———	———
Fungicide Program (F)	1	159.51	<0.0001
F × Cv	1	4.16	0.0461
F × Y	1	53.22	<0.0001
F × Y × Cv	1	1.21	0.2768
F × P	6	4.04	0.002
F × Cv × P	6	1.22	0.3081
F × Y × P	6	1.62	0.1591
F × Y × Cv × P	6	0.67	0.6704
Rep(F × Y × Cv × P), Error 4	56	———	———
Time (T)	5	729.64	<0.0001
T × Cv	5	17.58	<0.0001
T × Y	5	67.95	<0.0001
T × Y × Cv	5	33.21	<0.0001
T × P	30	3.17	<0.0001
T × Cv × P	30	1.99	0.0016
T × Y × P	30	2.25	0.0002
T × Y × Cv × P	30	1.52	0.0386
T × F	5	6.89	<0.0001
T × F × Cv	5	0.16	0.9757
T × Y × F	5	12.4	<0.0001
T × Y × Cv × F	5	1.57	0.1653
T × P × F	30	0.61	0.9508
T × Cv × P × F	30	0.66	0.9168
T × Y × P × F	30	1.15	0.2647
T × Y × Cv × P × F	30	0.6	0.9551
Error 5	560	———	———

Time by year by cultivar interaction effect

An interaction among time, year, and cultivar significantly ($p < 0.0001, f = 33.21$) affected mean TQ on nine of twelve dates in 2012 and 2013 (Table 2.1). During the months of June, August, September, October, and November of 2012, mean TQ of MiniVerde was 10, 10, 15, 24, and 11% greater, respectively, than Champion (Fig. 2.1). In 2013, during the months of June, July, August, and September, mean TQ of MiniVerde was 22, 14, 15, and 12% greater, respectively, than Champion (Fig. 2.1).

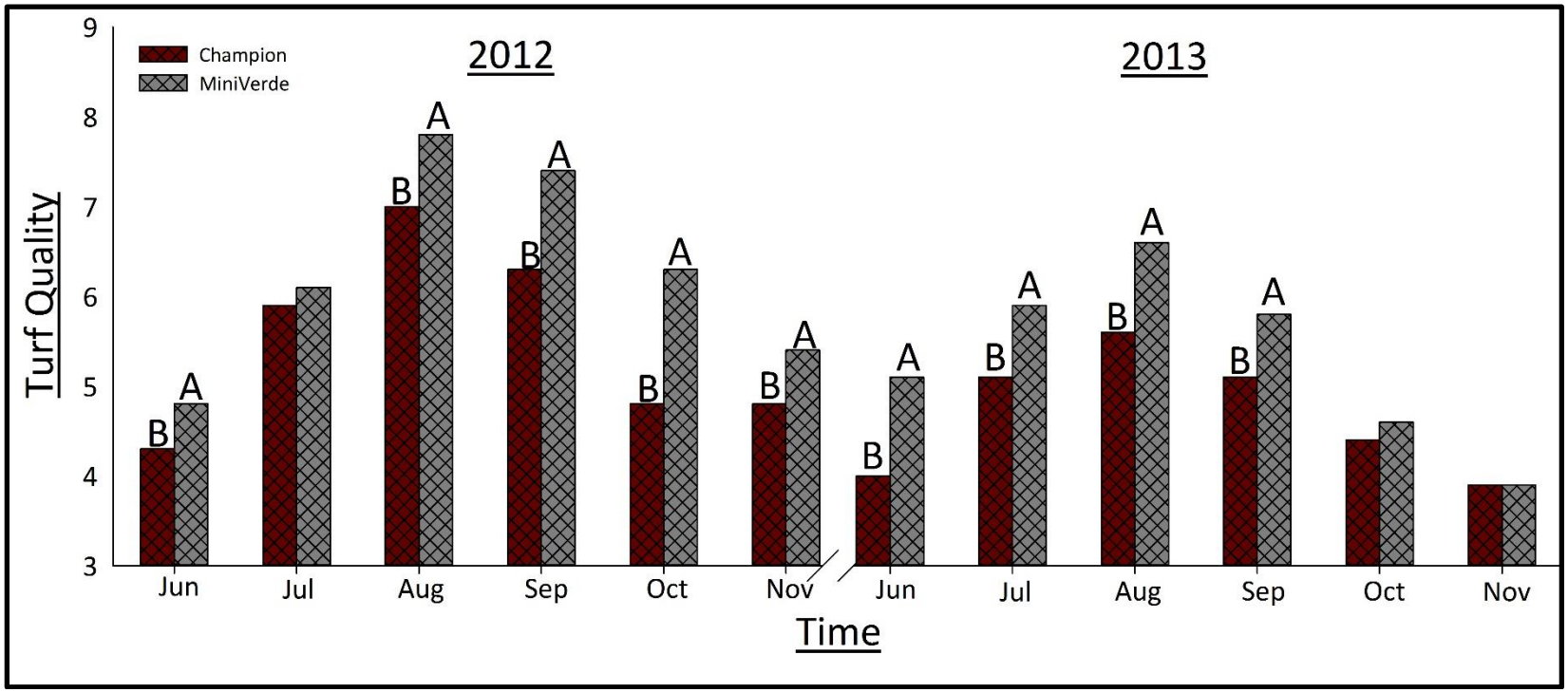

Figure 2.1 Mean turf quality (TQ) response to cultivar selection on ultradwarf bermudagrass managed with and without applications of seasonal fungicides in Starkville, MS from June to November in 2012 and 2013.

For each date, bars with different letters are significantly different at $p \leq 0.05$, based on differences of least squares means.

Time by year by fungicide program interaction effect

A significant ($p < 0.0001, f = 12.4$) interaction occurred among time, year, and fungicide program on eight of twelve dates in 2012 and 2013 (Table 2.1). During the months of September, October, and November of 2012, applications of seasonal fungicides increased mean TQ by 4, 4, and 6%, respectively (Fig. 2.2). In 2013, during the months of June, July, September, October, and November, seasonal fungicide applications resulted in mean TQ increases of 20, 12, 5, 16, and 5%, respectively (Fig. 2.2)

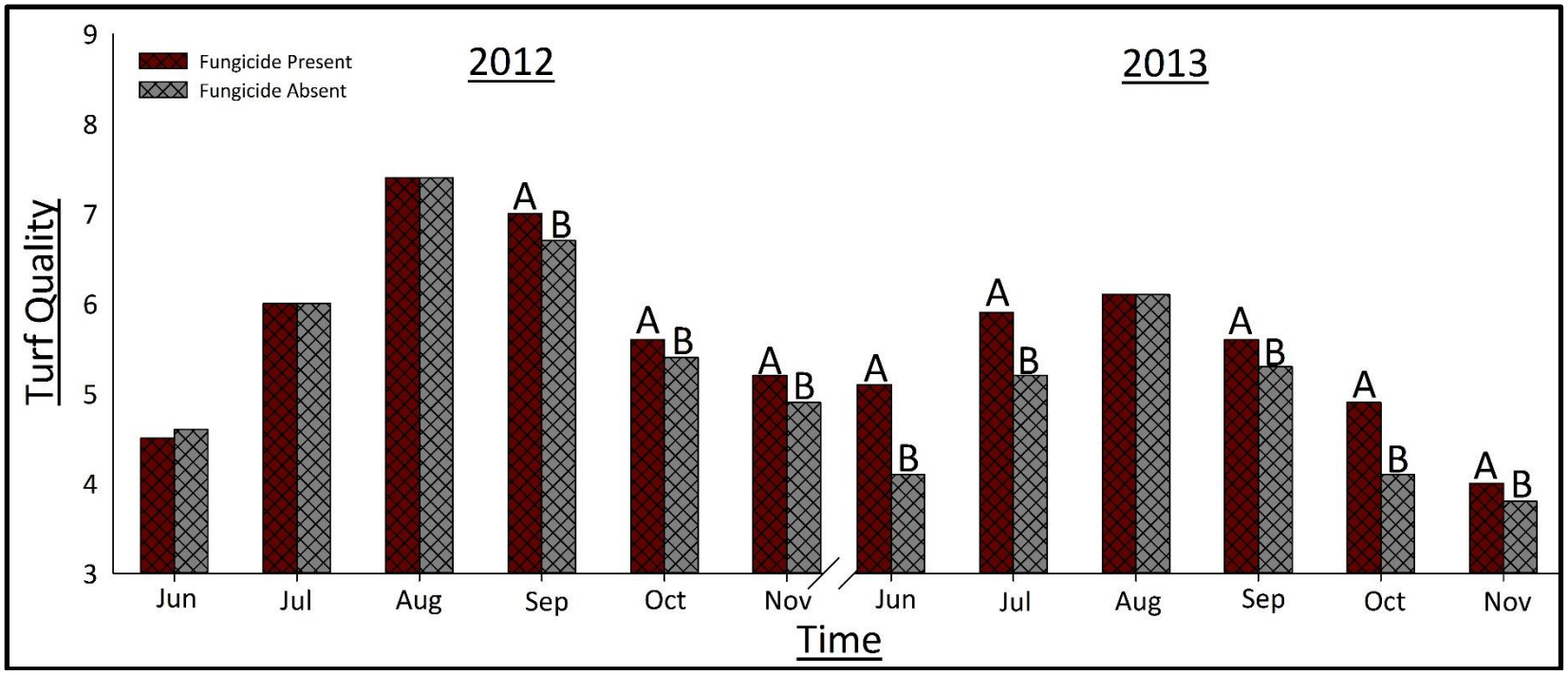

Figure 2.2 Mean turf quality (TQ) response to seasonal fungicide program on Champion and MiniVerde ultradwarf bermudagrass in Starkville, MS from June to November in 2012 and 2013.

For each date, bars with different letters are significantly different at $p \leq 0.05$, based on differences of least squares means.

Cultural practice main effect

Mean TQ was significantly ($p < 0.0001, f = 22.4$) affected by cultural practice treatments across all rating periods in 2012 and 2013 (Table 2.1). The VM control treatment was inferior to other treatments in the study, with respect to mean TQ (Fig. 2.3). Less-aggressive VM + SL, VM + SP, and VM + SC treatments significantly increased mean TQ values by 8, 8, and 9%, respectively, compared to VM (Fig. 2.3). The least-aggressive, infrequently applied SL, SP, and SC treatments were superior and resulted in significant mean TQ increases of 19, 17, and 19%, respectively, compared to VM (Fig. 2.3).

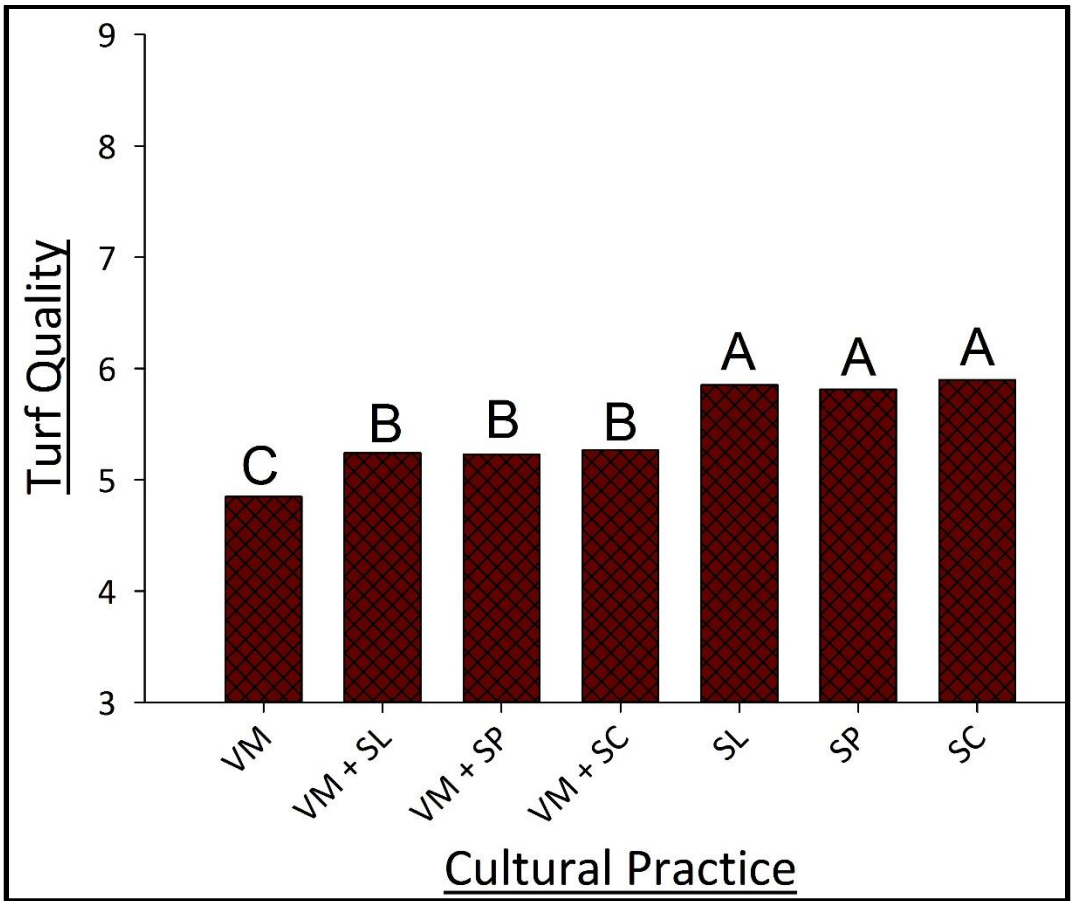

Figure 2.3 Mean turf quality (TQ) response to cultural management practices on Champion and MiniVerde ultradwarf bermudagrass managed with and without applications of seasonal fungicides in Starkville, MS in 2012 and 2013.

Bars with the same letter are not significantly different at $p \leq 0.05$, based on differences of least squares means.

Normalized Difference Vegetation Index

There were 1,008 observations for NDVI during the two-year study. In consideration of F-values, one interaction effect (time × year × cultivar) and two main effects (cultural practice) and (fungicide program) are discussed (Table 2.2).

Table 2.2 ANOVA for mean normalized difference vegetation index (NDVI) on Champion and MiniVerde ultradwarf bermudagrass in Starkville, MS from June to November in 2012 and 2013.

Source	DF	F-value	Pr > F
Rep	2	———	———
Cultivar (Cv)	1	2.16	0.2798
Rep × Cv, Error 1	2	———	———
Year (Y)	1	1.66	0.2667
Y × Cv	1	1.2	0.3341
Rep(Cv × Y), Error 2	4	———	———
Cultural Practice (P)	6	8.51	<0.0001
P × Cv	6	0.5	0.8083
P × Y	6	0.62	0.712
P × Y × Cv	6	0.67	0.6744
Rep(Y × Cv × P), Error 3	48	———	———
Fungicide Program (F)	1	18.98	<0.0001
F × Cv	1	10.89	0.0017
F × Y	1	1.72	0.1949
F × Y × Cv	1	0.19	0.6663
F × P	6	2.38	0.0408
F × Cv × P	6	1.51	0.1921
F × Y × P	6	0.38	0.8902
F × Y × Cv × P	6	0.15	0.9887
Rep(F × Y × Cv × P), Error 4	56	———	———
Time (T)	5	1438.25	<0.0001
T × Cv	5	9.18	<0.0001
T × Y	5	248.24	<0.0001
T × Y × Cv	5	5.41	<0.0001
T × P	30	1.81	0.0059
T × Cv × P	30	1.07	0.3732
T × Y × P	30	1.41	0.0762
T × Y × Cv × P	30	2.16	0.0004
T × F	5	2.14	0.0595
T × F × Cv	5	0.91	0.4722
T × Y × F	5	2.81	0.0161
T × Y × Cv × F	5	0.44	0.8181
T × P × F	30	0.33	0.9997
T × Cv × P × F	30	0.64	0.9355
T × Y × P × F	30	0.64	0.9355
T × Y × Cv × P × F	30	0.83	0.7237
Error 5	560	———	———

Time by year by cultivar interaction effect

An interaction among time, year, and cultivar significantly ($p < 0.0001, f = 5.41$) affected mean NDVI on five of twelve dates in 2012 and 2013 (Table 2.2). During the months of July 2012 and June and November 2013, mean NDVI of MiniVerde was 2, 3, and 4% greater, respectively, than Champion (Fig. 2.4). Mean NDVI of Champion was 2 and 5% greater than MiniVerde in September and October 2012, respectively (Fig. 2.4)

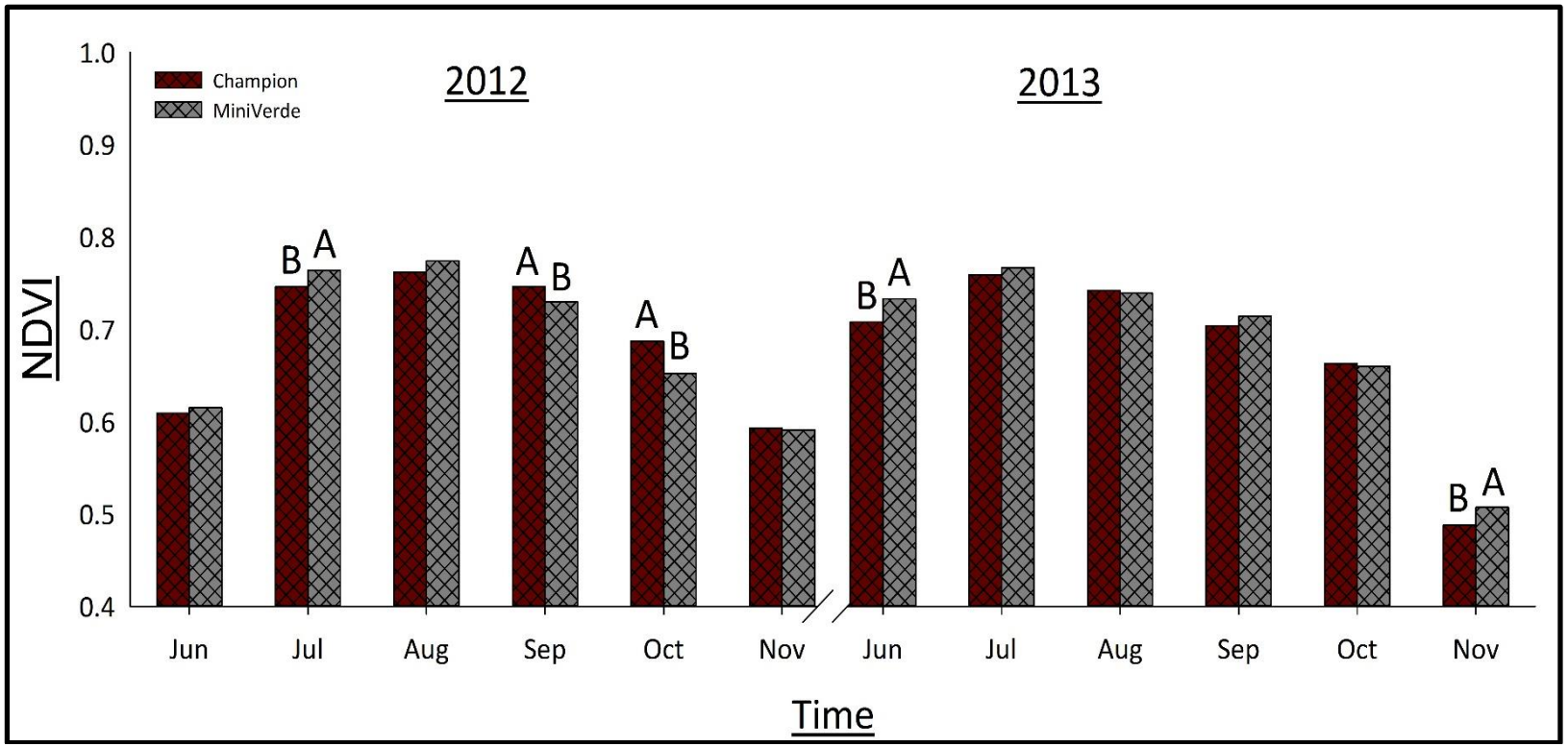

Figure 2.4 Mean normalized difference vegetation index (NDVI) response to cultivar selection on ultradwarf bermudagrass managed with and without applications of seasonal fungicides in Starkville, MS from June to November in 2012 and 2013.

For each date, bars with different letters are significantly different at $p \leq 0.05$, based on differences of least squares means.

Cultural practice main effect

Cultural practice treatments significantly ($p < 0.0001, f = 8.51$) affected mean NDVI across all rating periods in 2012 and 2013 (Table 2.2). The VM control treatment performed most poorly in the study, with respect to mean NDVI (Fig. 2.5). Less-aggressive VM + SL, VM + SP, and VM + SC treatments resulted in significant mean NDVI increases of 2, 3, and 2%, respectively, compared to VM (Fig. 2.5). The least-aggressive, infrequently applied SL, SP, and SC treatments were superior and increased mean NDVI values by 6, 5, and 5%, respectively, compared to VM (Fig. 2.5).

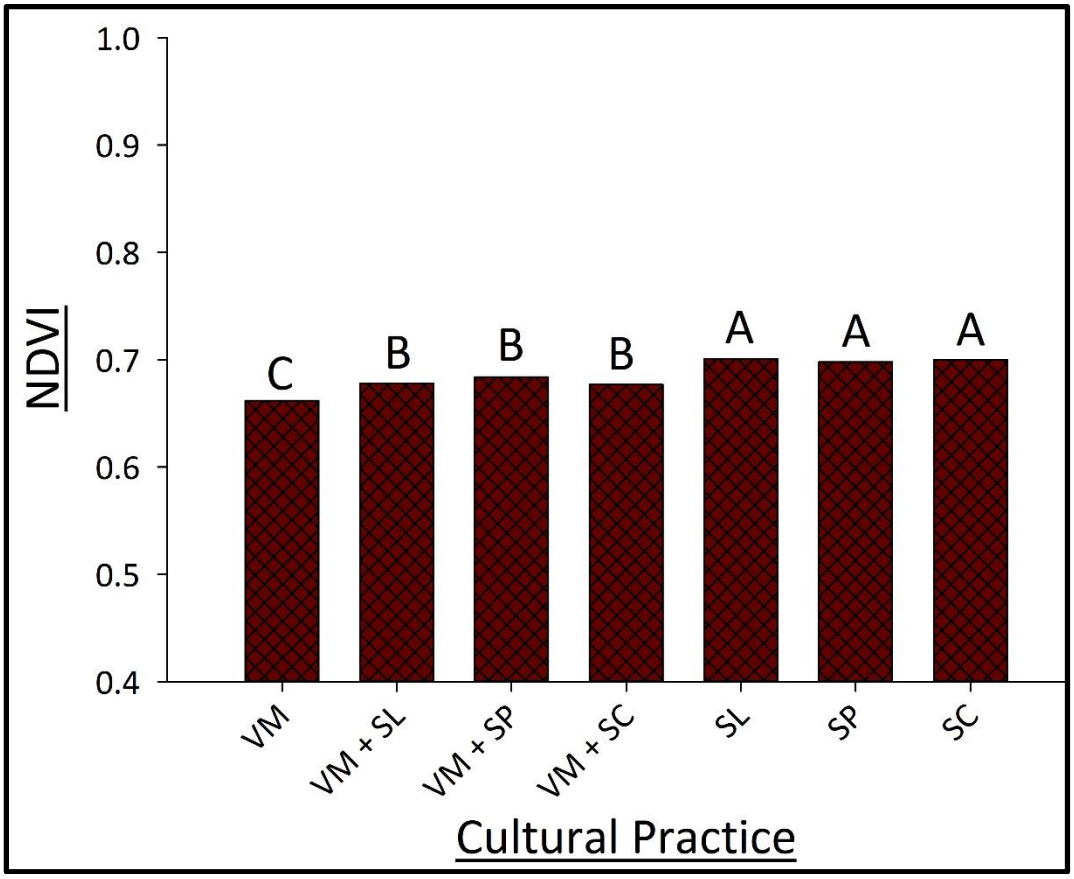

Figure 2.5 Mean normalized difference vegetation index (NDVI) response to cultural management practices on Champion and MiniVerde ultradwarf bermudagrass managed with and without applications of seasonal fungicides in Starkville, MS in 2012 and 2013.

Bars with the same letter are not significantly different at $p \leq 0.05$, based on differences of least squares means.

Fungicide program main effect

Fungicide program significantly ($p < 0.0001$, $f = 18.98$) affected mean NDVI (Table 2.2). Applications of seasonal fungicides increased mean NDVI by 1% compared to no fungicide treatments (Fig. 2.6).

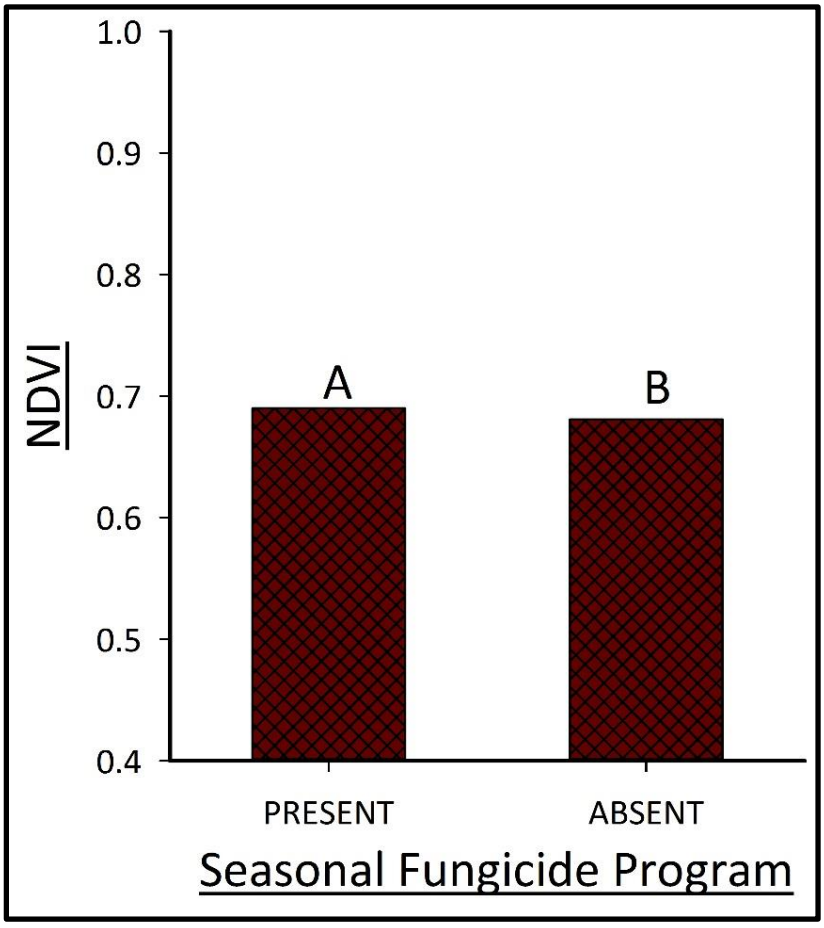

Figure 2.6 Mean normalized difference vegetation index (NDVI) response to seasonal fungicide program on Champion and MiniVerde ultradwarf bermudagrass in Starkville, MS in 2012 and 2013.

Bars with different letters are significantly different at $p \leq 0.05$, based on differences of least squares means.

Ball roll distance

During the two-year study, 840 stimpmeter readings were taken. In consideration of F-values, two interaction effects [(time × year) and (time × cultivar)] and one main effect (fungicide program) are presented (Table 2.3).

Table 2.3 ANOVA for mean ball roll distance (BRD) on Champion and MiniVerde ultradwarf bermudagrass in Starkville, MS from June to November 2012 and 2013.

Source	DF	F-value	Pr > F
Rep	2	———	———
Cultivar (Cv)	1	6.21	0.1304
Rep × Cv, Error 1	2	———	———
Year (Y)	1	29.55	0.0056
Y × Cv	1	1.29	0.3193
Rep(Cv × Y), Error 2	4	———	———
Cultural Practice (P)	6	1.36	0.2489
P × Cv	6	0.43	0.8562
P × Y	6	1.62	0.1621
P × Y × Cv	6	0.86	0.5331
Rep(Y × Cv × P), Error 3	48	———	———
Fungicide Program (F)	1	8.63	0.0048
F × Cv	1	0.46	0.4998
F × Y	1	0.67	0.4156
F × Y × Cv	1	0.27	0.6048
F × P	6	1.29	0.2787
F × Cv × P	6	2	0.0818
F × Y × P	6	1.3	0.2709
F × Y × Cv × P	6	3.2	0.0093
Rep(F × Y × Cv × P), Error 4	56	———	———
Time (T)	5	126.45	<0.0001
T × Cv	5	9.26	<0.0001
T × Y	5	100.12	<0.0001
T × Y × Cv	5	3.59	0.0068
T × P	30	0.95	0.5286
T × Cv × P	30	1.12	0.3129
T × Y × P	30	0.6	0.9322
T × Y × Cv × P	30	0.88	0.6349
T × F	5	1.05	0.3801
T × F × Cv	5	1.62	0.1676
T × Y × F	5	2.32	0.0566
T × Y × Cv × F	5	0.34	0.8504
T × P × F	30	1.49	0.0662
T × Cv × P × F	30	0.83	0.6968
T × Y × P × F	30	1.17	0.2625
T × Y × Cv × P × F	30	1.27	0.1789
Error 5	560	———	———

Time by year interaction effect

A significant ($p < 0.0001, f = 100.12$) interaction occurred between time and year in the study (Table 2.3). Although this interaction effect is highly significant and has a great F-value, it is not presented in detail because changes in BRD are expected to occur across different weather and environmental conditions (Fig. D.1) (Rist and Gaussoin, 1997).

Time by cultivar interaction effect

Mean BRD was also affected by a significant ($p < 0.0001, f = 9.26$) interaction between time and cultivar (Table 2.3). Champion outperformed MiniVerde at each time, with respect to mean BRD (Fig. 2.7). Mean BRD on Champion was 0.9, 87.2, 10.2, 9.3, and 14.8 cm greater than MiniVerde during the months of June, July, August, September, and October, respectively (Fig. 2.7)

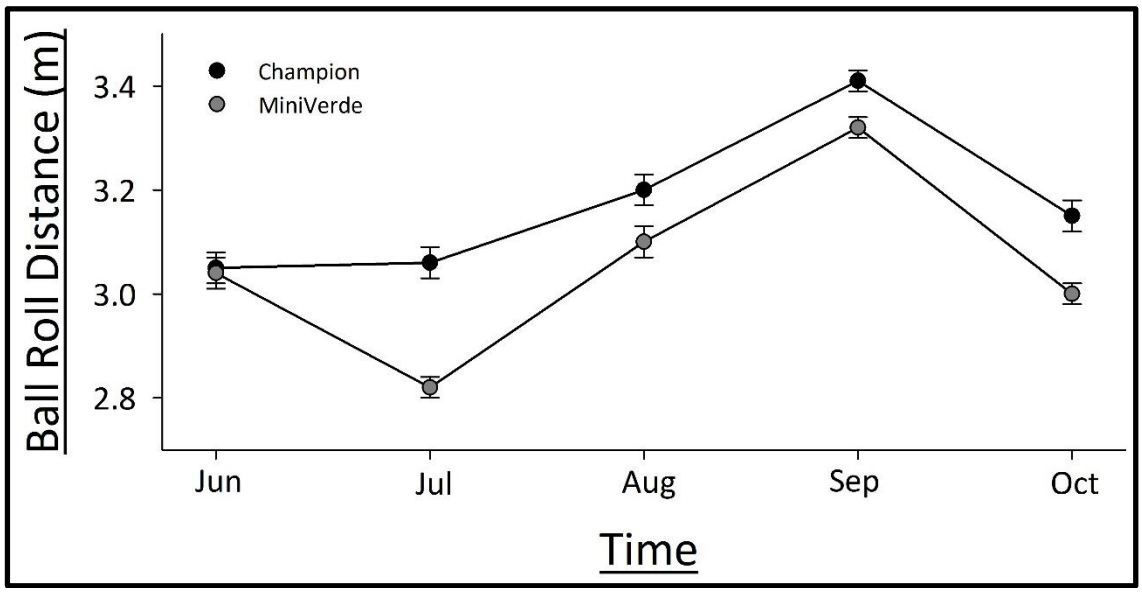

Figure 2.7 Mean ball roll distance (BRD) response to cultivar selection on ultradwarf bermudagrass managed with and without applications of seasonal fungicides in Starkville, MS From June to October in 2012 and 2013.

Fungicide program main effect

Fungicide program significantly (p = .0048, f = 8.63) affected mean BRD (Table 2.3). Applications of seasonal fungicides reduced mean BRD 3.0 cm compared to no fungicide treatments (Fig. 2.8).

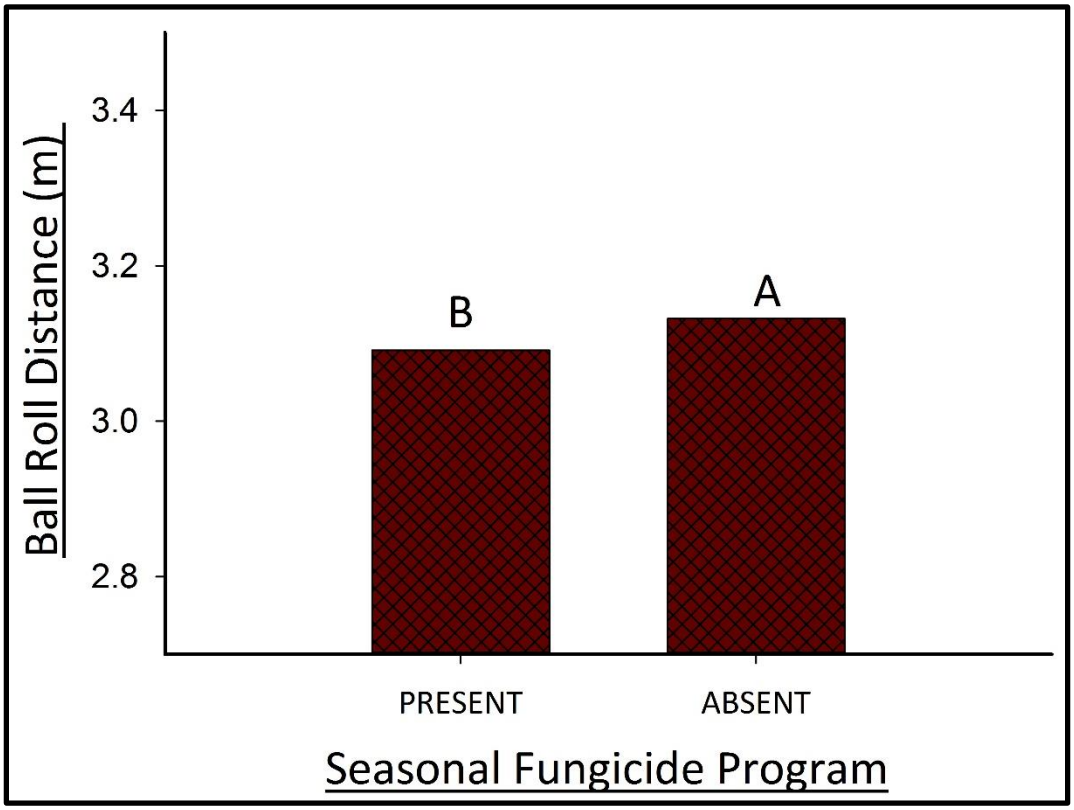

Figure 2.8 Mean ball roll distance (BRD) response to seasonal fungicide program on Champion and MiniVerde ultradwarf bermudagrass in Starkville, MS in 2012 and 2013.

Bars with different letters are significantly different at $p \leq 0.05$, based on differences of least squares means.

Surface Firmness

There were 1,008 observations for SF gathered during the two-year study. Two interaction effects [(cultivar × time) and (year × time)] and two main effects (cultural practice) and (fungicide program) significantly affected mean SF and exhibited F-values $\geq$ 4.0 (Table 2.4). These effects are discussed in following sections.

Table 2.5 ANOVA for mean surface firmness (SF) on Champion and MiniVerde ultradwarf bermudagrass in Starkville, MS from June to November 2012 and 2013.

Source	DF	F-value	Pr > F
Rep	2	———	———
Cultivar (Cv)	1	18.28	0.0506
Rep × Cv, Error 1	2	———	———
Year (Y)	1	240.01	0.0001
Y × Cv	1	5.94	0.0714
Rep(Cv × Y), Error 2	4	———	———
Cultural Practice (P)	6	11.2	<0.0001
P × Cv	6	2.23	0.0558
P × Y	6	1.69	0.1433
P × Y × Cv	6	1.41	0.2285
Rep(Y × Cv × P), Error 3	48	———	———
Fungicide Program (F)	1	17.13	0.0001
F × Cv	1	0	0.9607
F × Y	1	0.09	0.7706
F × Y × Cv	1	0.46	0.4986
F × P	6	1.5	0.1965
F × Cv × P	6	3.35	0.0069
F × Y × P	6	0.76	0.6047
F × Y × Cv × P	6	0.52	0.7886
Rep(F × Y × Cv × P), Error 4	56	———	———
Time (T)	5	52.69	<0.0001
T × Cv	5	5.71	<0.0001
T × Y	5	71.09	<0.0001
T × Y × Cv	5	2.02	0.0744
T × P	30	1.79	0.0068
T × Cv × P	30	0.99	0.485
T × Y × P	30	3.31	<0.0001
T × Y × Cv × P	30	1.07	0.3615
T × F	5	0.54	0.7437
T × F × Cv	5	0.58	0.7179
T × Y × F	5	0.65	0.6617
T × Y × Cv × F	5	1.06	0.3822
T × P × F	30	0.97	0.508
T × Cv × P × F	30	1.04	0.4094
T × Y × P × F	30	0.76	0.8213
T × Y × Cv × P × F	30	0.58	0.9672
Error 5	560	———	———

Time by cultivar interaction effect

An interaction between time and cultivar significantly ($p < 0.0001, f = 5.71$) affected mean SF (Table 2.4). MiniVerde was significantly firmer than Champion throughout the study, with respect to mean SF (Fig. 2.9). MiniVerde was 2, 5, 5, 6, 5, and 5% more firm than Champion during June, July, August, September, October, and November, respectively (Fig. 2.9).

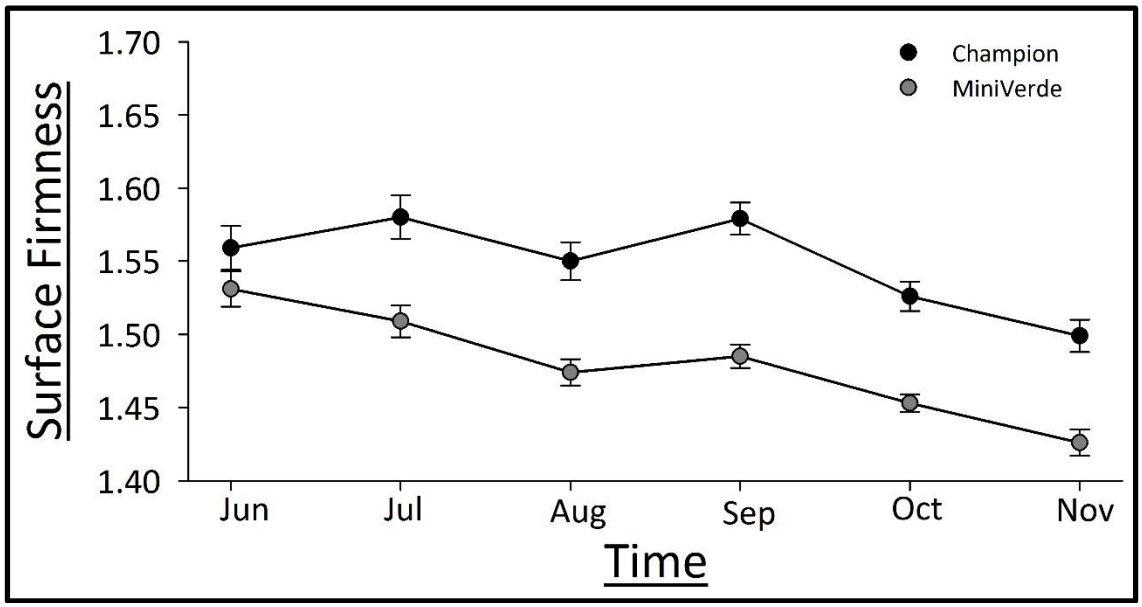

Figure 2.9 Mean surface firmness (SF) response to cultivars selection on ultradwarf bermudagrass managed with and without applications of seasonal fungicides in Starkville, MS from June to November in 2012 and 2013.

Time by year interaction effect

Mean SF of Champion and MiniVerde ultradwarf bermudagrass turf was significantly ($p < 0.0001, f = 71.09$) affected by an interaction between time and year (Table 2.4). With the exception of October, mean SF was significantly greater in 2013

than 2012. During the months of June, July, August, September, and November, mean SF was 12, 11, 7, 4, and 5% more firm in 2013 than 2012 (Fig. 2.10). Firmness was increased 9% from June to November in 2012 and 1% during the same period in 2013 (Fig. 2.10).

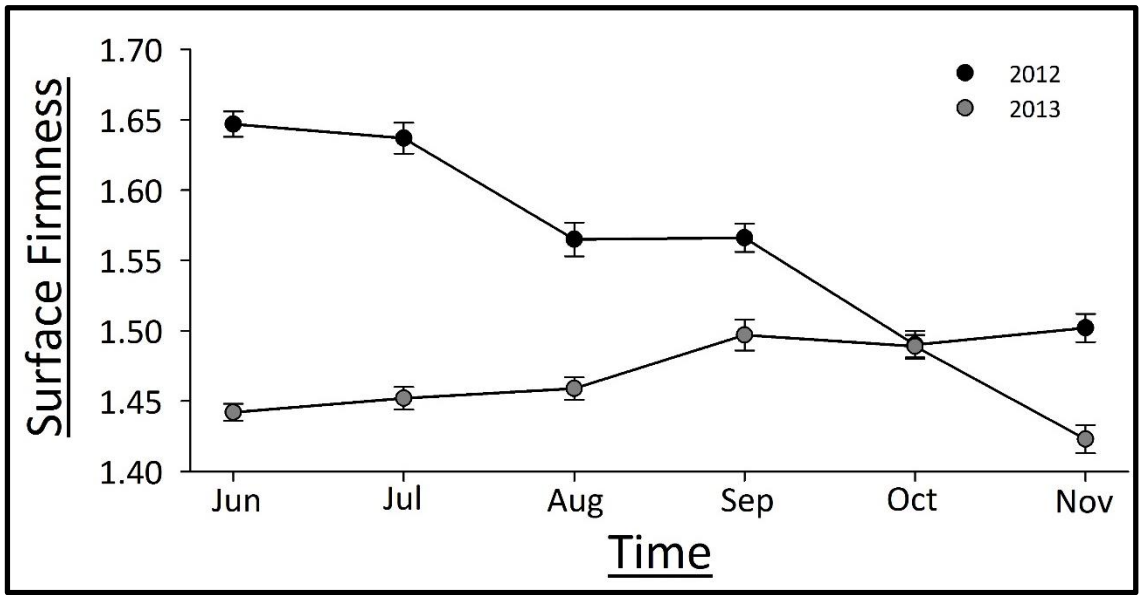

Figure 2.10 Mean surface firmness (SF) of Champion and MiniVerde ultradwarf bermudagrass managed with and without applications of seasonal fungicides in Starkville, MS from June to November in 2012 and 2013.

Cultural practice main effect

Cultural practice treatments significantly ($p < 0.0001$, $f = 11.2$) affected mean SF across all rating periods in 2012 and 2013 (Table 2.4). With respect to mean SF, VM treatments were the firmest, followed by the less-aggressive VM + SL and VM + SC treatments (Fig. 2.11). VM + SP treatments were significantly softer than VM, VM + SL, and VM + SC treatments, but firmer than the least-aggressive, infrequent SL, SP, and

SC cultural practice treatments (Fig. 2.11). In consideration of the SL, SP, and SC treatments, SL resulted in softest putting green surfaces with respect to mean SF (Fig 2.11).

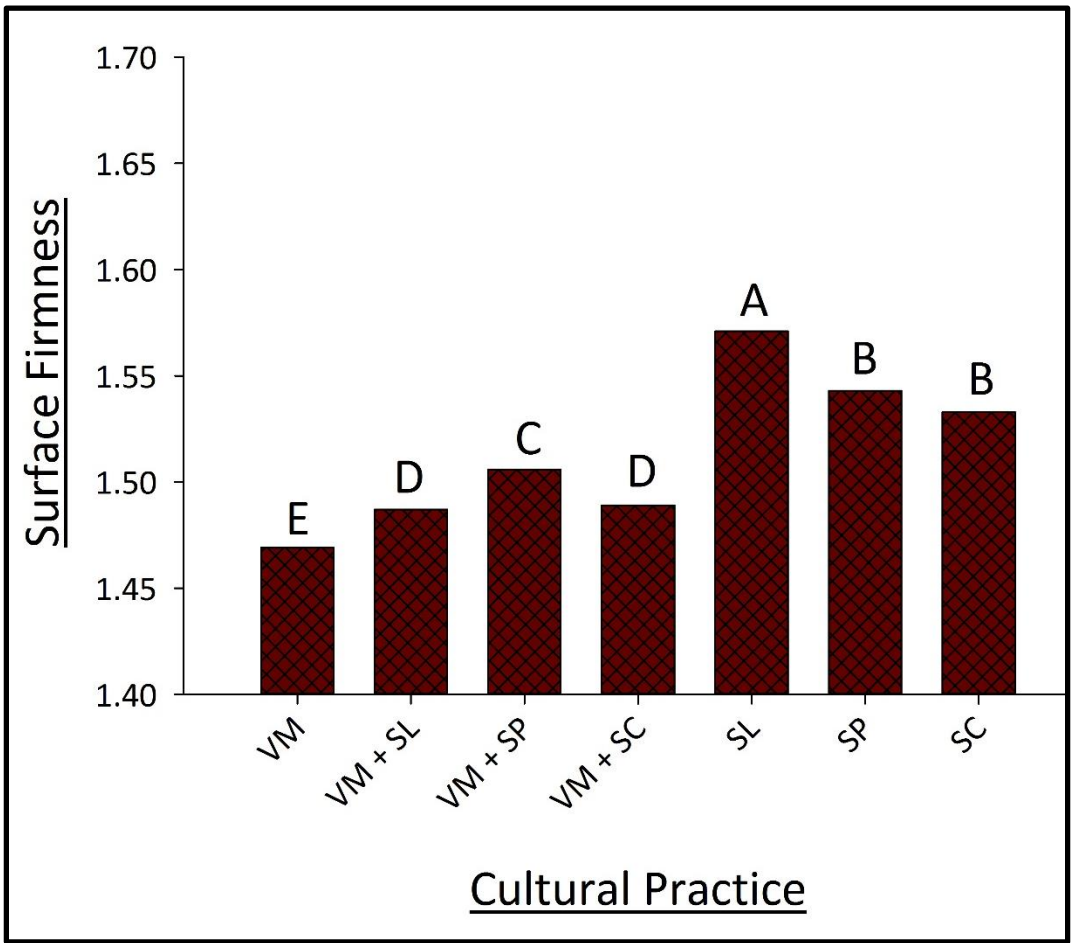

Figure 2.11 Mean surface firmness (SF) response to cultural management practices on Champion and MiniVerde ultradwarf bermudagrass managed with and without applications of seasonal fungicides in Starkville, MS in 2012 and 2013.

Bars with the same letter are not significantly different at $p \leq 0.05$, based on differences of least squares means.

Fungicide program main effect

Mean SF was significantly ($p < 0.0001, f = 17.13$) affected by levels of seasonal fungicide program (Table 2.4). Seasonal fungicide applications decreased firmness by 1% compared to no fungicide treatments (Fig. 2.12).

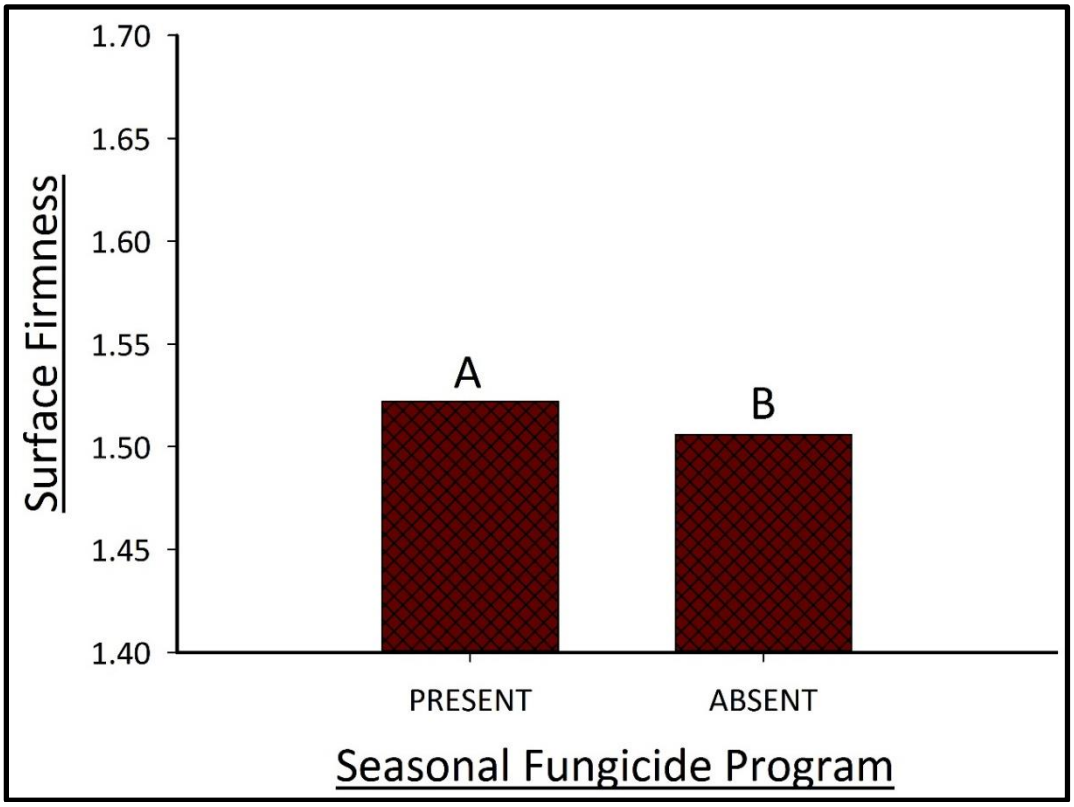

Figure 2.12 Mean surface firmness (SF) response to seasonal fungicide program on Champion and MiniVerde ultradwarf bermudagrass in Starkville, MS in 2012 and 2013.

Bars with different letters are significantly different at $p \leq 0.05$, based on differences of least squares means.

Discussion

Effects of cultural management practices, cultivar selection, and applications of seasonal fungicide programs on health and playability of ultradwarf bermudagrass turf were demonstrated in this study. Turf health was monitored as TQ and NDVI, and playability was assessed as BRD and SF. Cultural management practices were different with respect to TQ, NDVI, and SF whereas cultivar selection and applications of seasonal fungicides differed across TQ, NDVI, SF, and BRD.

Less-aggressive, infrequently applied SL, SP, and SC treatments were the best cultural management practices with respect to TQ and NDVI. Once monthly incorporations of SL, SP, and SC into weekly VM regimes resulted in TQ and NDVI values that were slightly decreased compared SL, SP, and SC treatments, but were better than TQ and NDVI values within VM treatments. In consideration of SF evaluations, SL, SP, and SC cultural management practices provided least desirable outcomes among all treatments in the study. As frequency of application increased with VM + SL, VM + SP, and VM + SC treatments, SF was significantly increased. Standard applications of weekly VM were the best cultural management practices with respect to SF. Although statistically significant, these SF values may not provide perceptible differences to golfers, and are possibly similar with respect to putting green playability.

In consideration of ultradwarf bermudagrass cultivars, MiniVerde provided superior TQ and SF results. Differences in SF are likely attributable to findings from a previous study, which demonstrated that Champion produces thatch at a more rapid rate than MiniVerde (Gray and White, 1999). Thatch is comprised of dead and decaying plant material, which decreases SF upon accumulation (Linde et al., 2011). Furthermore,

this provides a probable explanation for decreased TQ ratings because increased thatch accumulations lead to increased scalping, disease occurrence, and insect damage (Gray and White, 1999; Bevard, 2005; Vermeulen and Hartwiger, 2005; McCarty et al, 2007). Champion provided a greater BRD compared to MiniVerde; however, on average, differences were less than 15 cm. This does not present a practical effect on playability because golfers cannot detect green speed differences of this amount (Karcher et al., 2001). Results from NDVI evaluations were inconclusive, but suggest MiniVerde may have enhanced early season (June and July) turf color and Champion may have better late season (September and October) turf color. This concept has not been studied in detail, but may be of importance in scenarios where golf events are held early or late in the year. In such instances, a cultivar that has better green-up color or better color retention may be desirable.

Applications of seasonal fungicides resulted in significantly greater TQ and NDVI ratings compared to no seasonal fungicide applications. This difference was likely related to occurrence of foliar diseases such as dollar spot and leaf spot, which were readily observed August through November in 2012 and September through November in 2013 (Chapter III). Applications of seasonal fungicides resulted in decreased BRD values compared to no applications of seasonal fungicides. Although significant differences were observed, they were less than 15 cm, which is inconspicuous to golfers (Karcher et al., 2001). Therefore, practical differences in playability between levels of fungicide were not observed, with respect to BRD. A likely reasoning for the slight BRD increase among sub-plots that did not receive applications of seasonal fungicides is a disease related reduction in foliar plant material, resulting in reduced friction impact on

ball roll; a concept previously studied (Busey and Boyer, 1997). Sub-plots that received applications of seasonal fungicides were less firm than those that did not receive seasonal fungicide applications. Similar to differences between cultural management practices, the difference in depth of penetration between levels of fungicide was extremely narrow. While this variance is statistically significant, practical significance is improbable; and would unlikely warrant discontinued use of fungicides.

In consideration of plant health evaluations, TQ and NDVI, the best treatments were less-aggressive, infrequently applied SL, SP, and SC cultural practices. VM + SL, VM + SP, and VM + SC treatments had significantly reduced mean TQ and NDVI ratings compared to SL, SP, and SC, but were significantly better than VM treatments. Applications of seasonal fungicides significantly improved mean TQ and NDVI ratings, compared to no fungicide applications. MiniVerde had a significantly greater mean TQ than Champion; however, effects of cultivars on NDVI remain unclear.

With respect to BRD, none of the factors in this study had a practical influence. The differences between cultivars and seasonal fungicide treatments were minimal, at most; therefore, no conclusions were made for improved BRD. In consideration of SF, weekly applications of cultural practice treatments should be conducted to achieve most desirable SF levels. VM was the best treatment with respect to mean SF; however, less-aggressive VM + SL, VM + SP, and VM + SC treatments delivered comparable results. Additionally, with respect to mean SF, MiniVerde was a superior selection compared to Champion. There are other ultradwarf cultivars available for selection and this study provides evidence that SF differences may exist among them.

In consideration of playability and health of ultradwarf bermudagrass putting greens, management recommendations are made. Cultural practices should consist of weekly applications of VM with incorporations of less-aggressive SL, SP, or SC practices once each month. Fungicide applications should be conducted in consideration of host, pathogen, and environment, to protect against turfgrass diseases. Ultradwarf bermudagrass cultivar characteristics should be studied and considered prior to selection for planting on golf greens.

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CHAPTER III

EFFECTS OF CULTURAL MANAGEMENT PRACTICE, CULTIVAR SELECTION, AND SEASONAL FUNGICIDE PROGRAM ON FOLIAR DISEASES OF ULTRADWARF BERMUDAGRASS PUTTING GREENS

Abstract

Ultradwarf bermudagrass is used as golf course putting green turf throughout the southern U.S. Dollar spot, caused by *Sclerotinia homoeocarpa* F. T. Bennett, and leaf spot, caused by *Bipolaris cynodontis* (Marig.) Shoemaker are foliar diseases that affect health and aesthetics of ultradwarf bermudagrass putting greens. This two-year study evaluated the impacts of cultural management practices, cultivar selection, fungicides, and interactions of these factors on leaf spot and dollar spot of ultradwarf bermudagrass managed at 3.2 mm height of cut. Less-aggressive, infrequent cultural practices, including slice, spike, and scarify, applied once each month performed the best with respect to mean leaf spot severity (LSS) and dollar spot counts (DSC). Treatments consisting of incorporating these practices into weekly vertical mow regimes were not as good as the aforementioned treatments, but were better than standard vertical mow treatments. 'MiniVerde' displayed reduced LSS and DSC compared to 'Champion' throughout the study. Applications of fungicides significantly reduced mean LSS and DSC.

Introduction

Dollar spot, incited by *Sclerotinia homoeocarpa* F. T. Bennett, is a destructive, widely distributed disease of numerous turfgrass species in the U.S. (Walsh et al., 1999; Couch, 2000; Vargas, 1995; Smiley et al., 2005; Tomaso-Peterson and Perry, 2007). On ultradwarf bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy] golf greens and other closely mown turf (≤ 1.3 cm), dollar spot symptoms appear as sunken patches of blighted grass, usually less than 5 cm in diameter (Goodman and Burpee, 1991; Couch, 1995; Smiley et al., 2005). As dollar spot progresses, patches coalesce to form larger areas of collapsed, affected turf (Couch, 1995; Smiley et al., 2005; Tomaso-Peterson and Perry, 2007).

Leaf spot, caused by *Bipolaris cynodontis* (Marig.) Shoemaker, is another damaging foliar disease of bermudagrass turf (Couch, 1995; Smiley et al., 2005; Tomaso-Peterson and Young, 2010). Initial leaf symptoms appear as small, olive-green, pinpoint lesions, which advance to greenish black colored blotches (Couch, 1995; Tani and Beard, 1997; Smiley et al., 2005). Numerous infections can lead to wilting and chlorosis of leaf tissue (Couch, 1995; Tani and Beard, 1997; Smiley et al., 2005). Field symptoms appear as irregularly shaped expanses of straw-colored turf, ranging from 5 cm to 1 m in diameter, or greater (Couch, 1995; Tani and Beard, 1997; Smiley et al., 2005).

Historically, fungicide applications have supplied the most effective control for dollar spot of turfgrass (Walsh et al., 1999). However, issues of fungicidal efficacy for dollar spot control began in the 1960s with cadmium tolerance in *S. homoeocarpa* isolates (Cole et al., 1968). Additionally, resistance of *S. homoeocarpa* to benzimidazoles (Goldberg and Cole, 1973; Cole et al., 1974; Warren et al., 1974;

Detweiler et al., 1983; Smiley et al., 2005), demethylation inhibitors (Golembiewski et al., 1995), dicarboximides (Detweiler et al., 1983; Smiley et al., 2005), triazines (Nicholson et al., 1971), and heavy metal-based fungicides (Cole et al., 1968; Massie et al., 1968) have been documented.

Unlike dollar spot, limited information is available concerning chemical control for leaf spot of bermudagrass (Couch; 1995; Smiley et al., 2005). Trials conducted in Mississippi and Oklahoma disclosed inadequate success of numerous fungicides; only two of nine fungicides evaluated consistently reduced leaf spot severity (LSS) (Payne and Walker, 2014; Standish and Tomaso-Peterson, 2014; Tomaso-Peterson and Standish, 2014a; Tomaso-Peterson and Standish, 2014b).

Fungicide efficacy issues with dollar spot and leaf spot necessitate alternative control measures. Such management approaches include manipulative fertility inputs (Couch, 1995; Smiley et al., 2005; Tomaso-Peterson and Perry, 2007; Tomaso-Peterson and Young, 2010), reduced duration and quantity of leaf wetness (Couch, 1995; Williams et al., 1996; Turgeon, 2005; Giordano et al., 2012), and host resistant and tolerant cultivar selection (Couch, 1995; Jo, 2005; Bonos and Weibel, 2008; Tomaso-Peterson and Young, 2010). Restricted studies are available regarding tolerance and resistance among ultradwarf bermudagrass cultivars.

Ultradwarf bermudagrasses produce excessive thatch (Hollingsworth et al., 2000; Gregg and McCarty, 2004), which has been associated with increased disease pressure (Cornman, 1952; Musser, 1960; Murray and Juska, 1977; Bevard, 2005; Turgeon, 2005; Uddin et al., 2008). Popular management practices for thatch control include frequent vertical mowings and sand topdressing applications (Rowland, 2011; Lowe, 2013). Plant

injury is associated with vertical mowing (McCarty et al., 2007; Uddin et al., 2008); however, little is known about the effect it has on occurrence of foliar diseases of ultradwarf bermudagrass golf greens.

With consideration of current knowledge deficits, a study was conducted to assess leaf spot and dollar spot response to cultivar selection, cultural management practice, and fungicide applications on ultradwarf bermudagrass putting greens.

Materials and methods

Background management of research area

Research was conducted in Starkville, MS during 2012 and 2013 on four-year-old ultradwarf bermudagrass grown on a 90:10 sand:peat root zone mix with a pH of 6.5 (Fig. A.1). Research plots were managed according to golf course standards. Mowing events occurred daily at 3 to 4 mm height of cut with a Greensmaster® Flex™ 2100 walk-behind greens mower (The Toro Company, Bloomington, Minnesota, USA). Irrigation was supplied deep and infrequently to maintain moisture, promote vertical root growth, and avoid drought stress.

Fertility supplied nitrogen at 293 kg ha^{-1}, phosphorous at 98 kg ha^{-1}, and potassium at 342 kg ha^{-1} yr^{-1}. Primo MAXX® (trinexapac-ethyl, Syngenta, Greensboro, NC, USA) was applied at 0.026 kg a.i. ha^{-1} $week^{-1}$ during June, July, and August. Plots were core aerified with a Procore® 648 (The Toro Company, Bloomington, MN, USA) on 18 Jun 2012 and 16 Jul 2013. Cores, 7.6 to 10.2 cm length, were removed with 1 cm diameter hollow tines spaced 3.8 cm apart. Aerification events were followed by sand topdressing and brushed with a drag mat made of cocoa fiber to fill surface voids.

Experimental design and analysis

The study was arranged in a randomized complete block design with a split-plot constraint with three replications of each cultivar (Fig. A.2). Ultradwarf cultivars used were 'Champion' and 'MiniVerde'. Cultural practices were main-plot (2.1 × 2.4 m) factors and levels of fungicide were split plot (2.1 × 1.2 m) factors. Statistical analyses were conducted with the MIXED procedure in SAS v. 9.3 (SAS Institute Incorporated, Cary, NC, USA). Multiple comparisons were computed with least squares means for a given effect when the F-value was ≥ 4.0 and the F-ratio was significant at the 0.05 level. The F-value of 4.0 was chosen to systematically screen statistically significant results for practical significance, since the error degrees of freedom was large for many of the F-tests. Numerous high-order interactions were statistically significant, but from a practical standpoint, they were not meaningful.

Description and timing of treatment applications

There were two levels of fungicide in the study, either presence or absence of a seasonal fungicide spray regime. In 2012 and 2013, initial fungicidal applications were made early June with Honor (pyraclostrobin + boscalid, BASF, Research Triangle Park, NC, USA) at 0.512 kg a.i. ha^{-1} and 0.342 kg a.i. ha^{-1}, respectively. An application of Interface (iprodione + trifloxystrobin, Bayer Crop Science, Research Triangle Park, NC, USA) at 3.27 kg a.i. ha^{-1} and 0.198 kg a.i. ha^{-1}, respectively, was performed in July. A single application of Heritage (azoxystrobin, Syngenta, Greensboro, NC, USA) at 0.454 kg a.i. ha^{-1} was made in August. Chipco Signature (Aluminum tris, Bayer Crop Science, Research Triangle Park, NC, USA) tank-mixed with Fore (mancozeb, Dow AgroSciences, Indianapolis, IN, USA), each at 1.83 kg a.i. ha^{-1},was applied in

September, prior to a concluding fungicide application of Rubigan (fenarimol, Gowan Co., Yuma, AZ, USA) at 0.382 kg a.i. ha^{-1} in October.

Cultural practices were vertical mow (VM), vertical mow plus slice (VM + SL), vertical mow plus spike (VM + SP), vertical mow plus scarify (VM + SC), slice (SL), spike (SP), and scarify (SC). Cultural practice treatments were initiated 8 Jun 2012 and 12 Jun 2013 and terminated 14 Sep 2012 and 18 Sep 2013 (Table A.1, Fig. A.3)

Cultural practice treatments were applied with True-Surface® Greens Care Collection inserts (Turfline Incorporated, Moscow Mills, MO, USA). VM was included in the study as a control treatment to represent a management practice readily employed by golf course superintendents. VM + SL, VM + SP, and VM + SC were combination treatments included to determine the effect of once-monthly incorporated less-aggressive, alternative cultural practices on foliar disease occurrence. SL, SP, and SC treatments were applied once each month and were included to assess the effect of less frequent cultural practice applications on foliar disease occurrence.

VM treatments were conducted weekly with the Vacu-Cutter™ (Fig. A.4) insert in a bidirectional, perpendicular fashion at a depth of 4 mm and blades spaced 10 mm apart. The Vacu-Cutter™ insert is composed of 45 stainless steel blades, each having 11 tungsten carbide tips. VM + SL, VM + SP, and VM + SC treatments were applied the same as VM treatments except SL, SP, and SC applications were incorporated once each month, in a unidirectional fashion with the Deep Slicer (Fig. A.5) at a depth of 19 mm with blade spacing 40 mm apart, the Greens Spiker (Fig. A.6) at a depth of 19 mm and spikes spaced 60 mm apart, and the Vacu-Scarifier™ (Fig. A.7) at a depth of 9.5 mm

with 40 mm blade spacing, respectively. SL, SP, and SC treatments were applied once each month, in a unidirectional manner as previously described.

Data collection

Turf plots were evaluated for symptoms of leaf spot and dollar spot every 7 to 14 days, from June to November, during natural epiphytotics. Uniformity in natural infection of leaf spot and dollar spot across research plots eliminated the need for fungal inoculum. LSS was assessed by a visual estimation of percent symptomatic area (0 – 100%) within each subplot. DSC was physically assessed as number of dollar spot foci present in a given subplot.

Results

Leaf spot

Leaf spot was present during eight of the twelve rating months in the two-year study. Mean LSS for all treatments in July 2012 was 2.1 ± 0.6%, 3.4 ± 1.3% in August, 5.3 ± 1.4% in September, and 2.5 ± 0.7% in October. In 2013, it was 1.0 ± 0.5% in August, 6.3 ± 1.7% in September, 20.1 ± 2.5% in October, and 12.9 ± 2.2% in November. Multiple significant effects were observed for mean LSS (Table 3.1). However, in consideration of F-values, only two three-way interactions [(time × year × fungicide program) and (time × fungicide program × cultivar)] and one main effect (cultural practice) are deliberated (Table 3.1).

Table 3.1 ANOVA for mean leaf spot severity (LSS) on Champion and MiniVerde ultradwarf bermudagrass in Starkville, MS from June to November in 2012 and 2013.

Source	DF	F-value	Pr > F
Rep	2	———	———
Cultivar (Cv)	1	47.37	0.0205
Rep × Cv, Error 1	2	———	———
Year (Y)	1	25.76	0.0071
Y × Cv	1	1.31	0.3160
Rep(Cv × Y), Error 2	4	———	———
Cultural Practice (P)	6	8.85	<0.0001
P × Cv	6	1.49	0.2001
P × Y	6	2.72	0.0234
P × Y × Cv	6	0.34	0.9126
Rep(Y × Cv × P), Error 3	48	———	———
Fungicide Program (F)	1	56.59	<0.0001
F × Cv	1	11.83	0.0011
F × Y	1	7.89	0.0068
F × Y × Cv	1	1.63	0.2075
F × P	6	3.01	0.0128
F × Cv × P	6	0.2	0.9762
F × Y × P	6	1.14	0.3504
F × Y × Cv × P	6	1.35	0.2505
Rep(F × Y × Cv × P), Error 4	56	———	———
Time (T)	5	47.5	<0.0001
T × Cv	5	12.83	<0.0001
T × Y	5	49.05	<0.0001
T × Y × Cv	5	1.31	0.2588
T × P	30	3.09	<0.0001
T × Cv × P	30	1.54	0.0349
T × Y × P	30	3.49	<0.0001
T × Y × Cv × P	30	0.5	0.9883
T × F	5	17.56	<0.0001
T × F × Cv	5	7.55	<0.0001
T × Y × F	5	15.54	<0.0001
T × Y × Cv × F	5	1.47	0.1992
T × P × F	30	2.03	0.0012
T × Cv × P × F	30	1.96	0.0019
T × Y × P × F	30	2.51	<0.0001
T × Y × Cv × P × F	30	1.34	0.1111
Error 5	560	———	———

Time by year by fungicide program interaction effect

A significant interaction occurred among time, year, and fungicide program throughout the two-year study ($p < 0.0001$, $f = 15.54$) (Table 3.1). During the months of August, September, and October of 2012, seasonal fungicide applications resulted in 100, 87, and 96% mean LSS reductions, respectively (Table 3.2). In 2013, during the months of September, October, and November, seasonal fungicide applications resulted in mean LSS reductions of 85, 74, and 70%, respectively (Table 3.2)

Table 3.2 Mean leaf spot severity (LSS) as influenced by an interaction among time, year, and fungicide program on Champion and MiniVerde ultradwarf bermudagrass in Starkville, MS from June to November in 2012 and 2013.

2012	Jun	Jul	Aug	Sep	Oct	Nov
Fungicide Program	LSS					
Absent	0.0	3.5	6.8 a[†]	9.4 a	4.7 a	0.0
Present	0.0	0.8	0.0 b	1.2 b	0.2 b	0.0
2013						
Fungicide Program						
Absent	0.0	0.0	2.1	10.9 a	31.9 a	19.9 a
Present	0.0	0.0	0.0	1.6 b	8.4 b	6.0 b

[†]Within each column and year, means followed by different letters are significantly different at $p \leq 0.05$, based on differences of least squares means.

Time by fungicide program by cultivar interaction effect

Mean LSS was significantly affected by an interaction among time, fungicide program, and cultivar ($p < 0.0001$, $f = 7.55$) (Table 3.1). In the absence of a fungicide, compared to Champion, mean LSS was significantly less on MiniVerde at all times where significant differences occurred. During the months of August, September, and October, when a fungicide was not applied, MiniVerde exhibited 100, 100, and 22% less

mean LSS, respectively, than Champion (Table 3.3). Numerical differences occurred when a fungicide was applied, but were not statistically significant.

Table 3.3 Mean leaf spot severity (LSS) as influenced by an interaction among time, fungicide program, and cultivar selection on ultradwarf bermudagrass in Starkville, MS from June to November in 2012 and 2013.

Fungicide Absent	Jun	Jul	Aug	Sep	Oct	Nov
Cultivar	LSS					
Champion	0.0	3.5	8.8 a[†]	20.4 a	20.7 a	9.9
MiniVerde	0.0	0.8	0.0 b	0.0 b	16.2 b	10.0
Fungicide Present						
Cultivar						
Champion	0.0	0.8	0.0	2.8	5.0	2.7
MiniVerde	0.0	0.0	0.0	0.0	3.7	3.3

[†]Within each column and year, means followed by different letters are significantly different at $p \leq 0.05$, based on differences of least squares means.

Cultural practice main effect

Cultural practice treatments significantly affected mean LSS across all rating periods in 2012 and 2013 ($p < 0.0001$, $f = 7.55$) (Table 3.1). Mean LSS was greatest in VM treatments at 9.25 ± 1.7% (Fig. 3.1). Less-aggressive VM + SP, and VM + SC treatments resulted in significant mean LSS reductions of 44 and 56%, respectively, compared to the VM control treatment. Least-aggressive, infrequent SL, SP, and SC cultural practice treatments exhibited mean LSS reductions of 83, 75, and 80%, respectively, compared to the VM control treatment (Fig. 3.1).

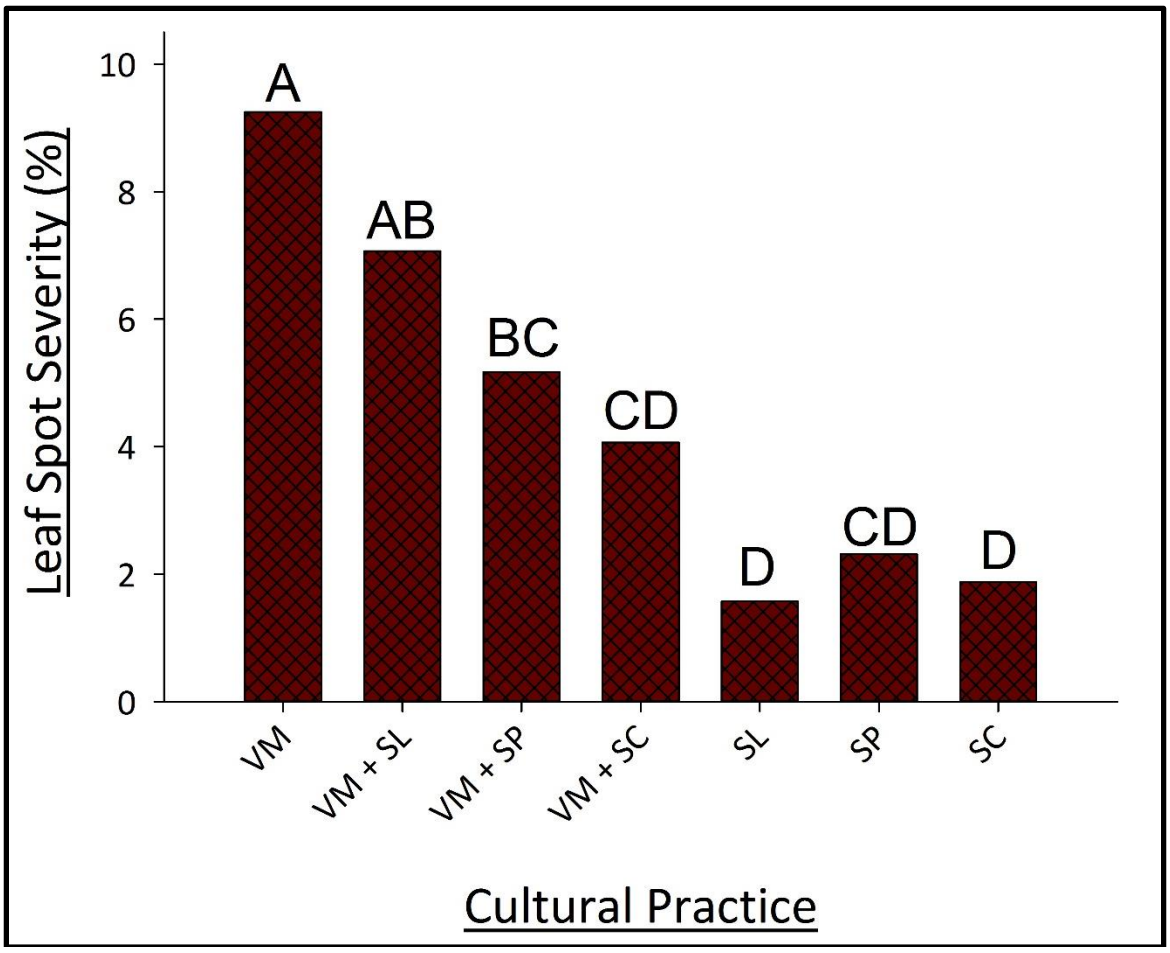

Figure 3.1 Mean leaf spot severity (LSS) response to cultural management practice on Champion and MiniVerde ultradwarf bermudagrass managed with and without applications of seasonal fungicides in Starkville, MS in 2012 and 2013.

Bars with the same letter are not significantly different at $p \leq 0.05$, based on differences of least squares means.

Dollar spot

Dollar spot symptoms were observed during the months of September through November 2012, and October and November 2013. In 2012, mean DSC for all treatments was 1.14 ± 0.45 in September, 7.79 ± 1.36 in October, and 6.99 ± 1.29 in November. In 2013, mean DSC was 5.95 ± 1.14 in October and 5.24 ± 1.09 in

November. In consideration of F-and *P*-values, one three-way interaction (time × year × cultivar) and two main effects (cultural practice) and (fungicide program) are discussed (Table 3.4).

Table 3.5 ANOVA for mean dollar spot count (DSC) on Champion and MiniVerde ultradwarf bermudagrass in Starkville, MS from June to November in 2012 and 2013.

Source	DF	F-value	Pr > F
Rep	2	———	———
Cultivar (Cv)	1	129.14	0.0077
Rep × Cv, Error 1	2	———	———
Year (Y)	1	2.91	0.1630
Y × Cv	1	9.43	0.0373
Rep(Cv × Y), Error 2	4	———	———
Cultural Practice (P)	6	6.39	<0.0001
P × Cv	6	1.19	0.3260
P × Y	6	0.59	0.7363
P × Y × Cv	6	1.19	0.3274
Rep(Y × Cv × P), Error 3	48	———	———
Fungicide Program (F)	1	45.54	<0.0001
F × Cv	1	2.97	0.0903
F × Y	1	0.01	0.9084
F × Y × Cv	1	2.00	0.1633
F × P	6	1.97	0.0862
F × Cv × P	6	1.89	0.0992
F × Y × P	6	1.14	0.3514
F × Y × Cv × P	6	0.45	0.8448
Rep(F × Y × Cv × P), Error 4	56	———	———
Time (T)	5	77.62	<0.0001
T × Cv	5	21.13	<0.0001
T × Y	5	1.44	0.2065
T × Y × Cv	5	6.62	<0.0001
T × P	30	7.03	<0.0001
T × Cv × P	30	1.34	0.0349
T × Y × P	30	0.72	0.1119
T × Y × Cv × P	30	1.34	0.1117
T × F	5	20.07	<0.0001
T × F × Cv	5	1.07	0.3759
T × Y × F	5	0.77	0.5718
T × Y × Cv × F	5	1.00	0.4144
T × P × F	30	0.98	0.5018
T × Cv × P × F	30	1.46	0.0545
T × Y × P × F	30	0.87	0.6646
T × Y × Cv × P × F	30	0.57	0.9677
Error 5	560	———	———

Time by year by cultivar interaction effect

A significant interaction occurred among time, year, and cultivar throughout the two-year study ($p < 0.0001, f = 6.62$) (Table 3.4). Mean DSC was significantly less on MiniVerde, compared to Champion, at all periods where differences occurred. During the months of September, October, and November of 2012, MiniVerde exhibited 100, 82, and 81% less mean DSC, respectively, than Champion (Fig. 3.2). In 2013, during the month of October, mean DSC was 65% less on Miniverde, compared to Champion (Fig 3.2).

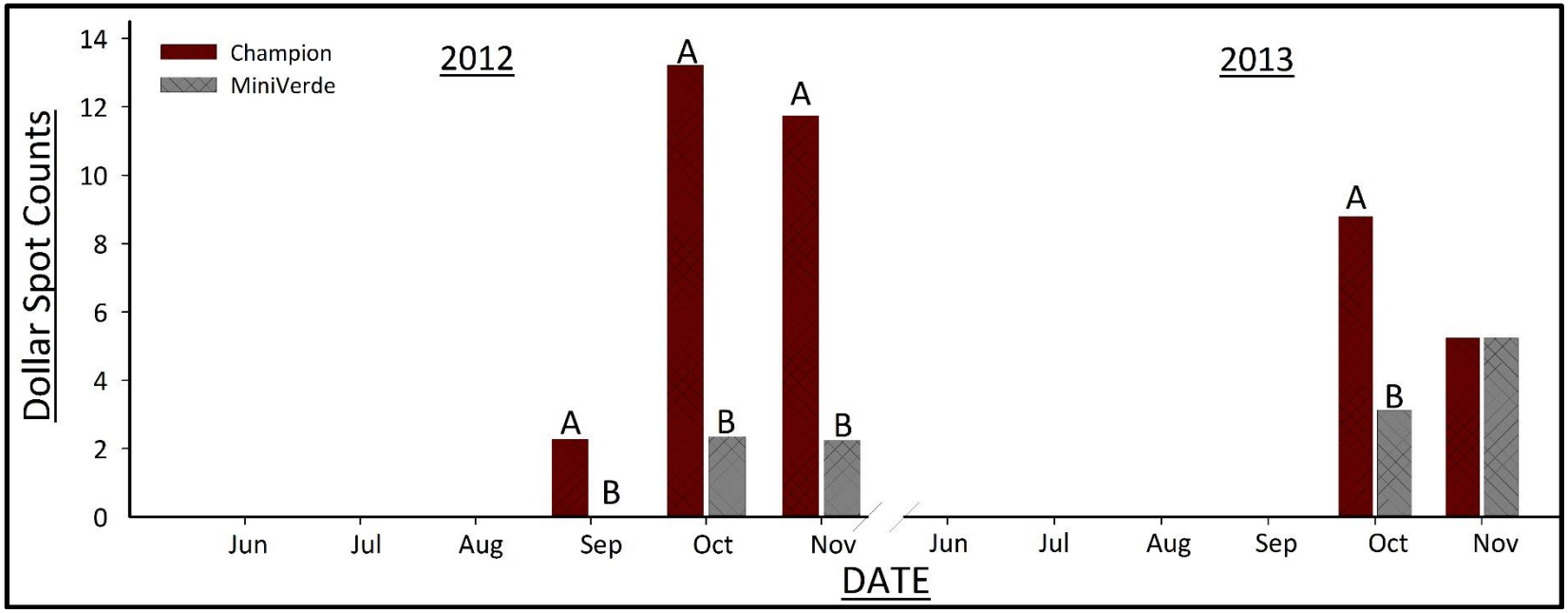

Figure 3.2 Mean dollar spot count (DSC) response to cultivar selection on ultradwarf bermudagrass managed with and without applications of seasonal fungicides in Starkville, MS from June to November in 2012 and 2013.

For each date, bars with different letters are significantly different at $p \leq 0.05$, based on differences of least squares means.

Fungicide program and cultural practice main effects

Fungicide program ($p < 0.0001, f = 45.54$) and cultural practice ($p < 0.0001, f = 6.39$) treatments independently and significantly affected mean DSC (Table 3.4). Seasonal fungicide applications resulted in 67% less mean DSC compared to no fungicide treatments (Fig. 3.3). All cultural practice treatments were better than the VM control treatment with respect to mean DSC (Fig. 3.4). Less-aggressive VM + SL, VM + SP, and VM + SC treatments resulted in significant mean DSC reductions of 31, 49, and 71%, respectively, compared to VM treatment (Fig 3.4). Least-aggressive, infrequent SL, SP, and SC cultural practice treatments exhibited mean DSC reductions of 93, 84, and 84%, respectively, compared to the VM control treatment (Fig. 3.4).

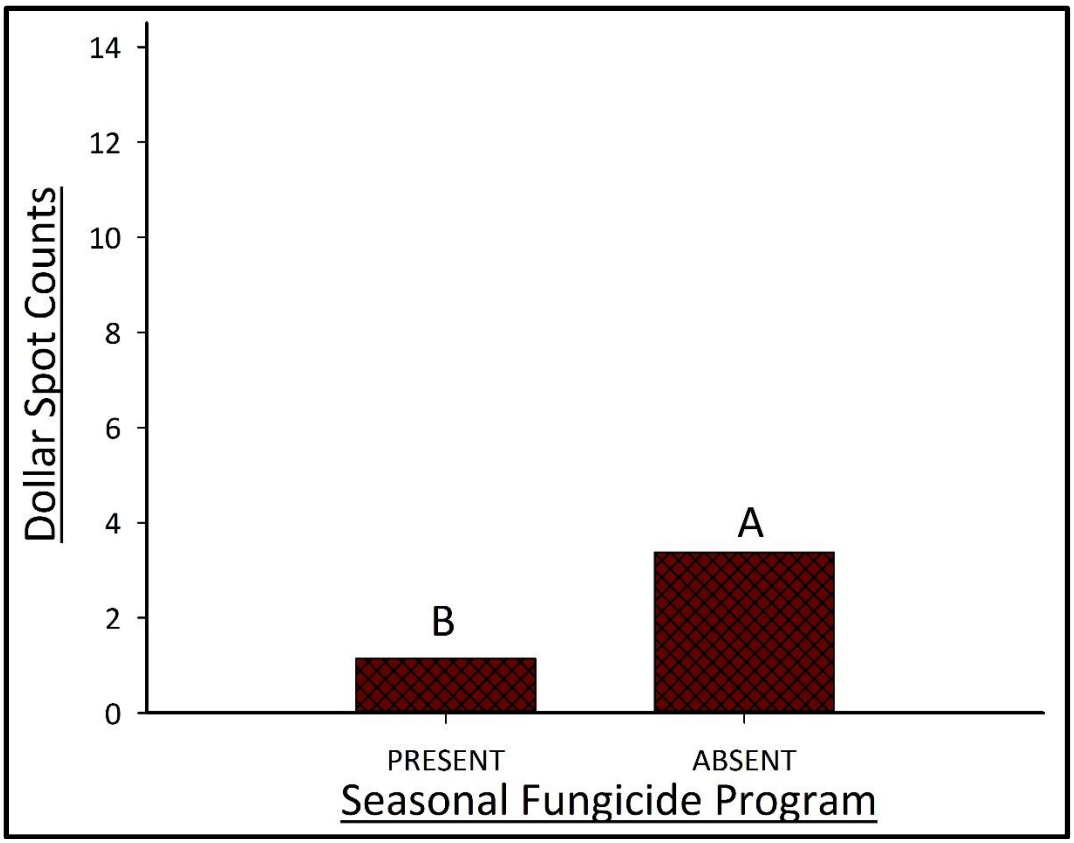

Figure 3.3 Mean dollar spot count (DSC) response to seasonal fungicide program on Champion and MiniVerde ultradwarf bermudagrass in Starkville, MS in 2012 and 2013.

Bars with different letters are significantly different at $p \leq 0.05$, based on differences of least squares means.

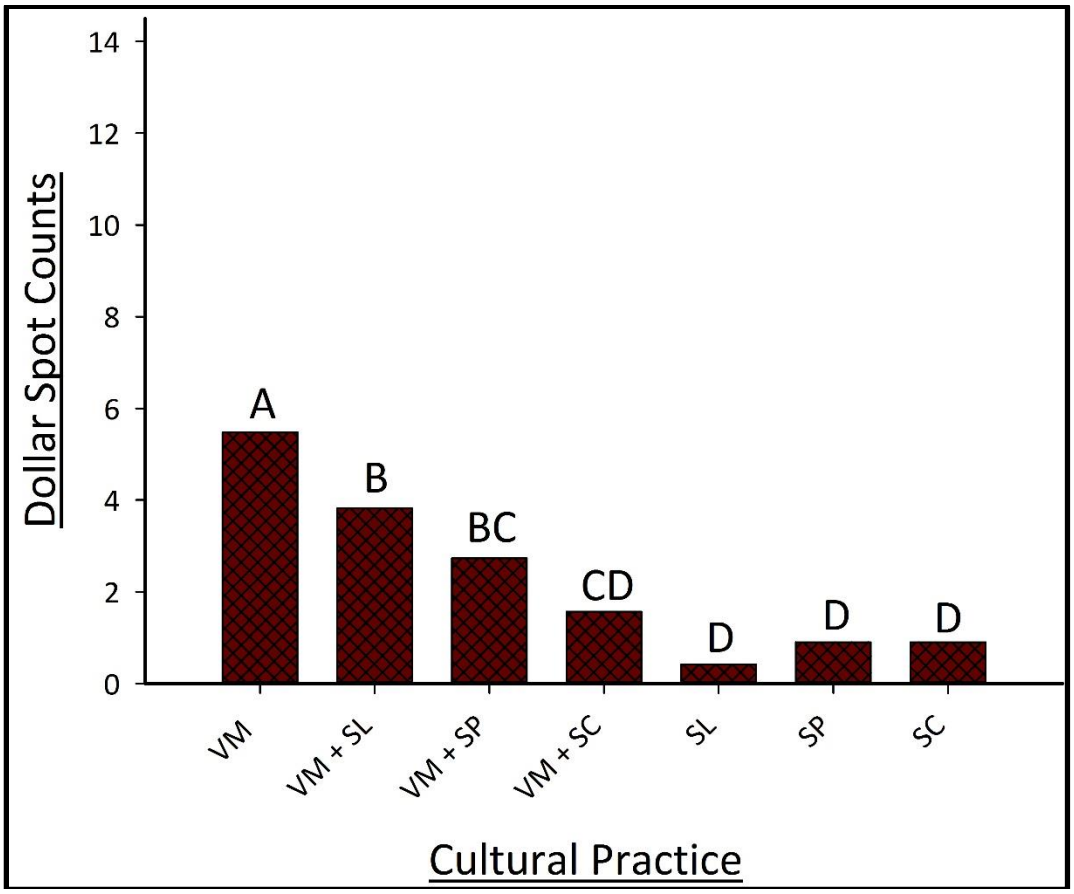

Figure 3.4 Mean dollar spot count (DSC) response to cultural management practice on ultradwarf bermudagrass turf managed at 3.2 mm in Starkville, MS.

Bars with the same letter are not significantly different at $p \leq 0.05$, based on differences of least squares means.

Discussion

Results from this study demonstrate effects of cultural management practices, cultivar selection, and applications of seasonal fungicide programs on foliar diseases of ultradwarf bermudagrass turf managed as a golf green. Limited information is available regarding cultural management and cultivar selection treatment effects on foliar diseases of ultradwarf bermudagrass; however, additional literature exists regarding their effects on other turf species.

Differences were observed between ultradwarf bermudagrass cultivars with respect to mean LSS and DSC. Compared to Champion, MiniVerde exhibited decreased LSS and DSC throughout the study. These findings indicate that MiniVerde may have an increased field tolerance to these two foliar diseases, compared to Champion. These cultivars have not been subjects of previous evaluations for foliar diseases; however, findings are similar to a study that demonstrated an increased susceptibility of 'FloraDwarf' to *B. cynodontis* when compared to 'TifEagle' and 'TifDwarf' cultivars (Brecht et al., 2007).

Findings indicated that standard cultural management practices consisting of weekly VM might not be ideal for foliar disease management of ultradwarf bermudagrasses. Weekly VM treatments were inferior to less-aggressive and less frequent alternative practices, with respect to mean LSS and mean DSC. Incorporating SL, SP, or SC treatments only once each month to VM practices significantly reduced mean LSS and mean DSC throughout the study. Once-monthly applications of SL, SP, or SC were consistently among the best treatments with respect to mean LSS and DSC

during the two-year study. These conclusions are consistent with previous research in other turfgrass-pathogen pathosystems (McCarty et al., 2005; Uddin et al., 2008).

Applications of a seasonal fungicide program significantly reduced mean LSS and DSC. However, alternative control measures must be considered in a valued disease management program. Two options to consider are cultivar selection and cultural management practice. MiniVerde was superior to Champion in this study and may be an option for alternative dollar spot and leaf spot control in ultradwarf bermudagrass management. In consideration of cultural management practices, currently used methods may be altered to provide improved foliar disease control. Results from this study suggest incorporating SL, SP, or SC practices once each month to reduce levels of foliar diseases. Furthermore, the best management practices, based on these findings, include SL, SP, or SC applications once each month.

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CHAPTER IV

IDENTIFICATION AND CHARACTERIZATION OF ECTOTROPHIC ROOT-INFECTING FUNGI ASSOCIATED WITH SUMMER DECLINE OF ULTRADWARF BERMUDAGRASS PUTTING GREENS

Abstract

Ultradwarf bermudagrass cultivars, in the Deep South region of the U.S., often exhibit symptoms of decline during late summer and early fall months. Root systems appear diminutive in size, brittle, and discolored and are frequently colonized with dark, runner hyphae that are characteristic of ectotrophic root-infecting (ERI) fungi. Based upon previous reports, spring dead spot (*Ophiosphaerella* spp.) and bermudagrass decline (*Gaeumannomyces graminis* var. *graminis*) are the only recognized diseases of ultradwarf bermudagrasses caused by ERI fungi. Observations from this study led to the theory that additional ERI fungi are associated with the late summer and early fall decline of ultradwarf bermudagrass. Due to laborious and inconclusive identification efforts associated with traditional diagnostic techniques, molecular-based strategies were employed to test the hypothesis. A multilocus sequence analysis was performed on the concatenated six-gene dataset of the representative Mississippi State University (MSU)-ERI isolates and related taxa. Phylograms were erected using combined consensuses from maximum likelihood and Bayesian inference analyses. The 26 MSU-ERI isolates included in the analyses were identified as *G. graminis* var. *graminis* (n=3), *G.*

paulograminis sp. nov. (n=7), *Magnaporthiopsis incrustans* (n=3), *M. hawaiiensis* sp. nov. (n=1), *M. cynodontis* sp. nov. (n=4), *M. taurocanis* sp. nov. (n=5), *Candidacolonium cynodontis* gen. nov. sp. nov. (n=2), and *Pseudophialophora cynodontis* sp. nov. (n=1). Studies were conducted to determine optimum temperatures for *in vitro* mycelium growth, which ranged from 26 to 30 C for novel ERI fungi.

Introduction

Ultradwarf bermudagrasses (*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy) are used throughout the southern U.S. as golf course putting green turfs. In this setting, these grasses exhibit fine leaf textures, short internodes, high shoot densities, and prostrate growing habits, all of which enable them to be tolerant of low mowing heights (Guertal et al., 2001). A major weakness of ultradwarf bermudagrasses is a decline of aesthetics and vigor during the late summer and early fall months in the Deep South. This occurrence has been labelled by the general term 'summer decline'.

Summer decline presents itself as a progression of symptomatology in field settings. During onset of decline, turfgrass plants lose color and slight loss of foliage is observed. As decline progresses, discolored areas become more pronounced and a greater degree of thinning in the turfgrass canopy can be observed. In extreme instances, large areas of turf are absent and bare soil remains (Fig. B.1). Root systems of affected plants are often brown to black in color, diminutive in size, brittle, and colonized with dark, runner hyphae (Figs. B2. And B.3). Upon closer inspection, simple and lobed hyphopodia and growth cessation structures have been noted (Figs. B.4 – B.6). Each of these structures is characteristic of ERI fungi (Clarke and Gould, 1993; Tredway, 2006).

Members of the ERI fungi are a soil-borne, root-infecting group of fungi encompassing several genera that affect gramineous hosts (Tredway, 2006). These fungi colonize plant roots via darkly pigmented, ectotrophic, robust runner hyphae (Tredway, 2006). Historically, this group of fungi has been reported to cause circular or patch-like dieback within turfgrass stands and is considered the cause of some of the most destructive turfgrass diseases in the U.S. (Landschoot and Jackson, 1990; Wetzel et al., 1996).

ERI fungi, as an assemblage, encompass several genera including *Gaeumannomyces* Arx and D. L. Olivier, *Ophiosphaerella* Spegazzini, and *Magnaporthiopsis* J. Luo and N. Zhang (≡ *Magnaporthe* R. A. Krause and R. K. Webster), which are housed in two families, Magnaporthaceae P. F. Cannon and Phaeosphaeriaceae M. E. Barr, of the Ascomycota. ERI fungi are responsible for a number of diseases on turfgrasses (Tredway, 2006). However, spring dead spot, caused by *O. herpotricha* J. C. Walker, *O. korrae* (J. C. Walker and A. M. Smith) R. Shoemaker and C. Babcock (≡ *Leptosphaeria korrae* J. C. Walker and A. M. Smith), and *O. narmari* (J. C. Walker and A. M. Smith) Wetzel, Hulbuert, and Tisserat (≡ *Leptosphaeria narmari* J. C. Walker and A. M. Smith), and bermudagrass decline, caused by *G. graminis* (Sacc.) Arx and D. Olivier var. *graminis*, are the only two diseases that affect bermudagrass. Additionally, *M. incrustans* (Landschoot and Jackson) J. Luo and N. Zhang (≡ *G. incrustans* Landschoot and Jackson) has been isolated from bermudagrass roots; however, its role in disease incitation has not been confirmed (Elliott, 1991).

Spring dead spot is among the most important diseases of hybrid bermudagrasses in North America and Australia (Smiley et al. 2005; Perry, 2008). Symptoms appear as

sunken, necrotic, bleached patches of turf that occur during the transitional period from winter dormancy to spring green-up (Smiley et al., 2005; Perry, 2008). Symptoms regularly recur in the same areas for consecutive years; however, size and shape of desiccated turf may be variable (Perry, 2008). The fungus produces dark brown, septate mycelial mats on roots and stolons of infected plants, and ascocarps are occasionally observed on dead tissues (Smiley et al., 2005).

Bermudagrass decline is particularly damaging to *Cynodon* spp. managed as golf and bowling greens (Smiley et al., 2005). Symptoms are most prevalent during warm to hot periods of summer and fall when humidity levels are high (Elliott, 1991; Smiley et al., 2005). Symptoms begin as irregular, chlorotic patches with chlorosis and necrosis first observed on lower leaves. Foliar lesions are not present. Root systems of affected plants are short and discolored with dark-colored lesions on the roots (Elliott and Landschoot, 1991). *G. graminis* var. *graminis* produces dark brown to black, runner hyphae on rhizomes, roots, and stolons of infected plants and, historically, has been identified by the presence of deeply lobed hyphopodia, which are used to penetrate host plant tissue (Smiley et al., 2005; Wetzel et al., 1996).

Two observations led to the concept that novel ERI fungal species are associated with the decline of ultradwarf bermudagrass in late summer and early fall months. Those observations were differences in field symptomatology and time of occurrence between summer decline and spring dead spot, as well as presence of chiroid and crenately lobed hyphopodia and growth cessation structures, which are not characteristic signs of *G. graminis* var. *graminis*. Accurate identifications of ERI fungi are generally difficult and time consuming via traditional diagnostic methods (Wetzel et al., 1996). With the

exception of the previously discussed deeply lobed hyphopodia of *G. graminis* var. *graminis*, the ectotrophs do not produce distinguishable features that allow for precise, timely identification (Wetzel et al., 1996).

ERI fungi must be isolated from infected plant material and formation of ascocarps must be induced for precise morphological identification. However, production of ascocarps is often unsuccessful due to incompatible mating types or attenuated biotypes. If correct mating types and biotypes are acquired, a period of four to ten weeks is required for the production of identification structures (Wetzel et al., 1996). Because of rigorous and rather demanding morphological identification measures, numerous molecular-based identification strategies have been studied for identification of ERI fungi.

The objective of this research was to employ molecular identification techniques to explore the hypothesis that novel ERI fungal species are associated with the decline of ultradwarf bermudagrasses during late summer and early fall months in the Deep South.

Materials and methods

Sample collection, fungal isolation, and long-term storage

Root materials of ultradwarf bermudagrass putting green samples (10.8 cm diameter) submitted to the Mississippi State University (MSU)-Plant Disease Diagnostic Laboratory were rinsed with distilled water to remove soil and organic material and microscopically screened for presence of dark, runner hyphae, a characteristic sign of ERI fungi. Roots that were colonized by these characteristic hyphal structures were cut into 5 mm sections and surface disinfested with a 0.6% sodium hypochlorite solution plus 1 ml 99% ethanol in a New Brunswick Scientific Excella E24 Incubator Shaker Series

(Thermo Fisher Scientific, Inc., Waltham, MA, USA) for 5 minutes. Disinfested root samples were rinsed three consecutive times with sterile-distilled water for 5 minutes each rinse. Surface disinfested roots were placed on sterile filter paper and allowed to dry for one hour under a laminar flow hood.

Dried root samples were plated, at random, onto modified strength potato dextrose agar (PDA) containing 6 g PDA and 15 g agar liter^{-1} amended with 100 mg chloramphenicol (dissolved in 2.5 ml ethanol) and 100 mg streptomycin sulfate (dissolved in 5.0 ml sterile dH_2O). Plated root tissues were incubated at 25 C until hyphal growth was observed protruding from the surface of the roots. Reduced contamination among fungi was ensured by transferring apical tips of hyphal strands to PDA (39 g liter^{-1}) at time of emergence. Axenic cultures were incubated at 25 C to allow for maturation of the colonies. Fungal cultures that exhibited similar morphological traits were grouped together and an illustrative sample from each group was chosen for characterization and identification.

Fungal cultures were prepared for long-term storage via previously described methods (Young et al., 2010; Gilley, 2013). Each isolate was grown on sterilized glass fiber filter paper (Thermo Fisher Scientific, Inc., Waltham, MA, USA) placed atop PDA (39 g liter^{-1}). Sub-cultured fungi were incubated at 25 C under 24 hour fluorescent light for 14 days. Fully colonized glass fiber filter paper was removed from PDA, dried in a laminar flow hood, cut into 3 mm^2 pieces, placed in 60 mm petri dishes wrapped with parafilm (Sigma-Aldrich, Inc., Saint Louis, MO, USA), and subsequently stored at –20 C for long-term storage.

Genomic DNA extraction, PCR amplification, and sequencing

Genomic deoxyribonucleic acid (gDNA) was extracted from vibrant mycelium of the representative pure colony (n = 139) of each group of morphologically similar fungi via manufacturer's instruction for the Fungi/Yeast Genomic DNA Isolation Kit (Norgen Biotek Corp, Thorold, ON, Canada). Purity and concentration of extracted gDNA was determined via a NanoDrop 2000/2000c (Thermo Fisher Scientific, Inc., Waltham, MA, USA) by monitoring light absorbance at wavelengths of 260 nm and 280 nm. Desirable samples were stored at –20 C to –80 C.

Preliminary screenings were conducted on all representative isolates in the study to ensure their relatedness to known members of Magnaporthaceae and Phaeosphaeriaceae. The screening process included amplification and sequencing of the internal transcribed spacer (ITS) region of the ribosomal ribonucleic acid (rRNA) genes and conducting a BLAST (Altschul et al., 1990) analysis on the resultant sequence (Table B.1). Desirable samples (n = 29) were subjected to additional amplification and sequencing of genes described by Zhang et al. (2011) to provide a more robust analysis of their genetic relationships to known fungal species. Additional genes included the 28S large subunit (LSU) rRNA gene, the 18S small subunit (SSU) rRNA gene, a deoxyribonucleic acid (DNA) replication licensing factor gene for minichromosome maintenance complex component 7 (*MCM7*), the largest subunit of RNA polymerase II gene (*RPBI*), and the translation elongation factor 1-alpha gene (*TEF1*).

Polymerase chain reaction (PCR) was conducted with GoTaq® PCR Core Systems (Promega Corp, Madison, WI, USA) in a MyCycler™ Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using cycling conditions adopted and modified

from Zhang et al. (2011). ITS, LSU, SSU, and *TEF1* PCR consisted of 1 µl of template DNA at a concentration of 10 ng μl^{-1} and 49 µl aliquots of a master mix containing 4 µl of 25 mM magnesium chloride ($MgCl_2$), 10 µl of 5X GoTaq® Reaction Buffer, 1 µl of 10 mM deoxynucleotide triphosphates (dNTPs), 0.25 µl of GoTaq® DNA Polymerase, 3 µl of forward and reverse primers, each at 5 µM concentration, and 27.75 µl of nuclease and protease free water (Sigma-Aldrich, Inc., Saint Louis, MO, USA).

Optimization of *MCM7* and *RPB1* PCR resulted in different master mixes than those used for ITS, LSU, SSU, and *TEF1*. Individual *MCM7* and *RPB1* PCR consisted of 1 µl of template DNA at a concentration of 10 ng μl^{-1} and 49 µl aliquots of a master mix containing 4 µl of 25 mM $MgCl_2$, 10 µl of 5X GoTaq® Reaction Buffer, 1 µl of 10 mM dNTPs, 0.25 µl of GoTaq® DNA Polymerase, 8 µl of forward and reverse primers, each at 5 µM concentration, and 17.75 µl of nuclease and protease free water.

Cycling conditions for ITS, LSU, SSU, *TEF1*, and *MCM7* included a primary denaturation step at 95 C for 2 minutes followed by 35 cycles consisting of denaturation for 1 minute at 95 C, annealing for 1 minute at 57 C, and extension for 1 minute at 72 C. Cycles were concluded with a final extension period of 10 minutes at 72 C. Cycling conditions for *RPB1* were identical except for an increased annealing temperature, from 57 C to 58 C, and a ramp rate of 0.2 C sec^{-1} between the 58 C annealing temperature and the 72 C extension temperature. Amplified PCR products were visualized in 1.0% agarose gels (Figs. B7 – B.14) prior to sequencing, which was conducted at MWG Operon (Eurofins Genomics, Huntsville, AL, USA). PCR primers used for amplification reactions were defined by previous research and are provided in Table 4.1.

Table 4.1 Molecular markers and descriptions of the six loci used in molecular phylogenetic studies.

Locus[†]	Primer name	Primer Sequence (5'–3')	GC content (%)[‡]	# of characters[§]	Reference
ITS	ITS5	GGAAGTAAAAGTCGTAACAAGG	40.9	711	White et al., 1990
	ITS4	TCCTCCGCTTATTGATATGC	45.0		White et al., 1990
LSU	LS1	GTACCCGCTGAACTTAAGC	52.6	946	Rehner and Samuels, 1995
	LR5	TCCTGAGGGAAACTTCG	52.9		Rehner and Samuels, 1990
SSU	SR1R	TACCTGGTTGATTCTGCCAGT	47.6	1112	Vilgalys and Hester, 1990
	SR7	GTTCAACTACGAGCTTTTTAA	33.3		Vilgalys and Hester, 1990
MCM7	MCM7–709	ACIMGIGTITCVGAYGTHAARCC	45.7	625	Schmitt et al., 2009
	MCM7–1348	GAYTTDGCIACICCIGGRTCWCCCAT	51.3		Schmitt et al., 2009
RPB1	RPB1–Ac	GARTGYCCDGGDCAYTTYGG	58.3	836	Matheny et al., 2002
	RPB1–Cr	CCNGCDATNTCRTTRTCCATRTA	42.8		Castlebury et al., 2004
TEF1	EF1–983F	GCYCCYGGHCAYCGTGAYTT	61.7	1099	Carbone and Kohn, 1999
	EF1–2218R	ATGACACCRACRGCRACRGTYTGYAT	50.0		Rehner and Buckley, 2005
Combined				7329	

[†]Genetic loci, ITS = Internal Transcribed Spacer, LSU = Large Subunit, SSU = Small Subunit, *MCM7* = Minichromosome Maintenance Complex Component 7, *RPB1* = RNA Polymerase II, *TEF1* = Translation Elongation Factor 1-alpha.
[‡]Percentage of nitrogenous bases in oligonucleotide sequence that are either guanine or cytosine.
[§]Number of nucleotide characters amplified with the corresponding primer set.

Sequence data and phylogenetic analyses

Individual gene sequence alignments were conducted with MUSCLE (Edgar, 2004) with default parameter settings as applied in MEGA6 (Tamura et al., 2013) and adjusted manually where necessary. Analyses were performed on individual gene alignments and datasets of concatenated gene alignments using *Cryphonectria parasitica* (Murrill) M. E. Barr as an outgroup taxon. Phylogenetic trees were estimated by Bayesian inference (BI) as implemented in MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), and maximum likelihood (ML) as implemented in MEGA6. The *p*-distance, which presents the percentage of differences among nucleotides of two sequences, was used to calculate pairwise distances.

BI analyses were projected with the Markov chain Monte Carlo method in MrBayes v3.1.2 by running four simultaneous chains for 1×10^7 generations, sampling every 100 generations, and using default priors. Once average standard deviation of split frequencies remained < 0.01 and likelihood scores reached an asymptotic value, a given run was considered to have reached convergence. All trees sampled prior to convergence were discarded. Support for nodes and parameter estimates were evaluated from a majority consensus of the last 75,000 trees post-convergence.

ML analyses were conducted with the appropriate evolutionary nucleotide substitution model. One thousand pseudoreplicates were employed to assess branch support.

Optimum temperature for *in vitro* growth

Optimal growing temperatures were determined via temperature-regulated incubation studies as described by Perry (2008). Representative isolates were sub-cultured by transferring 2 mm diameter hyphal plugs from 10-day old cultures grown on PDA (39 g liter^{-1}) amended with streptomycin sulfate and chloramphenicol. Hyphal plugs were placed, mycelium-side down, onto fresh PDA (39 g liter^{-1}) and incubated at temperatures of 18, 25, 32, and 38 C in darkness (Figs. B.15 and B.16). Each isolate was replicated three times at each temperature. Following 7-day incubation, measurements of radial growth (mm) were taken at four perpendicular points for each replicate (Figs. B.17 – B.20). Radial growth measurements of representative isolates were analyzed using the GLM procedure of SAS (SAS version 9.3; SAS Institute, Cary, NC, USA), and optimal growth temperatures were determined by the following quadratic equation: $x = (-1 \times B_1) / (2 \times B_2)$, where B_1 = linear term and B_2 = quadratic term.

Cultural characteristics

Fungal isolates were grown on PDA for cultural characteristic studies. Colony colors were named according to the Inter-Society Colour Council of America (I.S.C.C.) and the National Bureau of Standards (N.B.S) (Rayner, 1970). Microscopic measurements and examinations were taken from slides of fungi mounted in either sterile distilled water or lactophenol.

Results

Fungal isolates

Twenty-six ERI fungal isolates from ultradwarf bermudagrass were used in this study (Figs. B.21 – B.72). Three isolates identified as *M. poae*, TAP35 (Figs. B.73 and B.74) and TAP41 (Figs. B.75 and B.76) from creeping bentgrass (*Agrostis stolonifera* L.) and SPKBG7 (Figs. B.77 and B.78) from Kentucky bluegrass (*Poa pratensis* L.), were provided by L. P. Tredway to serve as recognized, positive controls (Tredway, 2006). Forty isolates from previous studies performed by Thongkantha et al. (2009), Zhang et al. (2011), Luo and Zhang (2013), and Luo et al. (2014) were utilized as reference taxa. Six DNA sequences were included for each isolate, resulting in 414 sequences in the combined dataset for this study (Table 4.2).

Table 4.2 Species name, Isolate ID, and GenBank accession numbers for fungi used in molecular phylogenetic studies.

Species Name	Isolate ID	ITS	LSU	SSU	*MCM7*	*RPB1*	*TEF1*
Buergenerula spartinae Kohlm. and R.V. Gessner	ATCC 22848[†]	JX134666	DQ341492	DQ341471	JX134706	JX134720	JX134692
Candidacolonium cynodontis[‡] P.L. Vines and M. Tomaso–Peterson	HP24–3	KJ855497	KM401637	KP129316	KP007345	KP268919	KP282703
C. cynodontis	HP38–4	KJ855498	KM401638	KP129317	KP007344	KP268920	KP282704
Cryphonectria parasitica (Murrill) M.E. Barr	EP155	——————————Genome data, Joint Genome Institute——————————					
Gaeumannomyces cylindrosporus D. Hornby, Slope, Gutter. and Sivan.	CBS 610.75	JX134667	DQ341494	DQ341473	JX134707	JX134721	JX134693
G. graminis var. *avenae* (E.M. Turner) Dennis	CBS 187.65	JX134668	JX134680	JX134655	JX134708	JX134722	JX134694
G. graminis var. *graminis* (Sacc.) Arx and D.L. Oliver	M33	JF710374	JF414896	JF414871	JF710392	JF710442	JF710411
G. graminis var. *graminis*	M53	JF414847	JF414897	JF414872	JF710393	JF710443	JF710418
G. graminis var. *graminis*	M54	JF414848	JF414898	JF414873	JF710394	JF710444	JF710419
G. graminis var. *graminis*	CBS 235.32	JX134669	JX134681	DQ341476	JX134709	JX134723	JX134695
G. graminis* var. *graminis	AD1–2	KJ855487	KM401627	KP129306	KP007334	KP268909	KP282693
G. graminis* var. *graminis	GSGC15–3	KJ855494	KM401634	KP129313	KP007341	KP268916	KP282700
G. graminis* var. *graminis	GSGC15–4	KJ855495	KM401635	KP129314	KP007342	KP268917	KP282701
G. graminis var. *tritici* J. Walker	R3–111a–1	——————————Genome data, Broad Institute——————————					
G. graminis var. *tritici*	M55	JF414850	JF414900	JF414875	JF710395	JF710445	JF710420
G. paulograminis P. L. Vines and M. Tomaso-Peterson	DR1–4	KJ855491	KM401628	KP129307	KP007335	KP268910	KP282697
G. paulograminis	DR6–4	KJ855492	KM401629	KP129308	KP007336	KP268911	KP282698

Table 4.2 (continued)

Species Name	Isolate ID	ITS	LSU	SSU	*MCM7*	*RPB1*	*TEF1*
G. paulograminis	DR10–3	KJ855488	KM401630	KP129309	KP007337	KP268912	KP282694
G. paulograminis	DR12–1	KJ855489	KM401631	KP129310	KP007338	KP268913	KP282695
G. paulograminis	DR13–1	KJ855490	KM401632	KP129311	KP007339	KP268914	KP282696
G. paulograminis	OW4–4	KJ855501	KM401641	KP129320	KP007333	KP268923	KP282707
G. paulograminis	RS7–1	KJ855507	KM401647	KP129326	KP007350	KP268929	KP282713
M. cynodontis P. L. Vines and M. Tomaso-Peterson	HCC3–4	KJ855496	KM401636	KP129315	KP007343	KP268918	KP282702
M. cynodontis	RS3–1	KJ855505	KM401645	KP129324	KP007348	KP268927	KP282711
M. cynodontis	RS5–5	KJ855506	KM401646	KP129325	KP007349	KP268928	KP282712
M. cynodontis	RS7–2	KJ855508	KM401648	KP129327	KP007351	KP268930	KP282714
M. hawaiiensis P. L. Vines and M. Tomaso-Peterson	KR10–6	KJ855499	KM401639	KP129318	KP007357	KP268921	KP282705
M. incrustans (Landsch. and N. Jacks.) J. Luo and N. Zhang	M35	JF414843	JF414892	JF414867	JF710386	JF710437	JF710412
M. incrustans	M51	JF414846	JF414895	JF414870	JF710389	JF710440	JF710417
M. incrustans	LC8–6	KJ855500	KM401640	KP129319	KP007330	KP268922	KP282706
M. incrustans	RRFMV14–5	KJ855504	KM401644	KP129323	KP007331	KP268926	KP282710
M. incrustans	WW3–5	KJ855515	KM401655	KP129334	KP007332	KP268937	KP282721
M. panicorum J. Luo and N. Zhang	CM2S8	KF689643	KF689633	KF689593	KF689603	KF689613	KF689623
M. panicorum	CM7m9	KF689645	KF689635	KF689595	KF689605	KF689615	KF689625
M. panicorum	CM9m11	KF689646	KF689636	KF689596	KF689606	KF689616	KF689626
M. panicorum	CM10s2	KF689644	KF689634	KF689594	KF689604	KF689614	KF689624
M. poae (Landsch. and N. Jacks.) J. Luo and N. Zhang	ATCC 64411	——————————Genome data, Broad Institute——————————					
M. poae	M47	JF414836	JF414885	JF414860	JF710390	JF710433	JF710415
M. poae	SPKBG7	KJ855510	KM401650	KP129329	KP007353	KP268932	KP282716

Table 4.2 (continued)

Species Name	Isolate ID	ITS	LSU	SSU	*MCM7*	*RPB1*	*TEF1*
M. poae	TAP35	KJ855511	KM401651	KP129330	KP007358	KP268933	KP282717
M. poae	TAP41	KJ855512	KM401652	KP129331	KP007354	KP268934	KP282718
M. rhizophila (D.B. Scott and Deacon) J. Luo and N. Zhang	M22	JF414833	JF414882	JF414857	JF710383	JF710431	JF710407
M. rhizophila	M23	JF414834	JF414883	JF414858	JF710384	JF710432	JF710408
M. taurocanis P. L. Vines and M. Tomaso-Peterson	GSGC10–2	KJ855493	KM401633	KP129312	KP007340	KP268915	KP282699
M. taurocanis	RRFCHMP1–3	KJ855502	KM401642	KP129321	KP007346	KP268924	KP282708
M. taurocanis	RRFMV10–2	KJ855503	KM401643	KP129322	KP007347	KP268925	KP282709
M. taurocanis	TPC4–5	KJ855513	KM401653	KP129332	KP007355	KP268935	KP282719
M. taurocanis	TPC5–3	KJ855514	KM401654	KP129333	KP007356	KP268936	KP282720
Nakataea oryzae (Catt.) J. Luo and N. Zhang	M21	JF414838	JF414887	JF414862	JF710382	JF710441	JF710406
N. oryzae	M69	JX134672	JX134684	JX134658	JX134712	JX134726	JX134698
N. oryzae	M71	JX134673	JX134685	JX134659	JX134713	JX134727	JX134699
Omnidemptus affinis P.F. Cannon and Alcorn	ATCC 200212	JX134674	JX134686	JX134660	JX134714	JX134728	JX134700
Ophioceras commune Shearer, J.L. Crane and W. Chen	M91	JX134675	JX134687	JX134661	JX134715	JX134729	JX134701
O. commune	M92	JX134676	JX134688	JX134662	JX134716	JX134730	JX134702
O. dolichostomum	CBS 114926	JX134677	JX134689	JX134663	JX134717	JX134731	JX134703
O. leptosporum (S.H. Iqbal) J. Walker	CBS 894.70	JX134678	JX134690	JX134664	JX134718	JX134732	JX134704
Pseudohalonectria lignicola Minoura and T. Muroi	M95	JX134679	JX134691	JX134665	JX134719	JX134733	JX134705

Table 4.2 (continued)

Species Name	Isolate ID	ITS	LSU	SSU	*MCM7*	*RPB1*	*TEF1*
Pseudophialophora cynodontis P.L. Vines and M. Tomaso–Peterson	RW3–4	KJ855509	KM401649	KP129328	KP007352	KP268931	KP282715
P. eragrostis J. Luo and N. Zhang	CM12M9	KF689648	KF689638	KF689598	KF689608	KF689618	KF689628
P. eragrostis	CM20m5–2	KF689647	KF689637	KF689597	KF689607	KF689617	KF689627
P. panicorum J. Luo and N. Zhang	CM3m7	KF689652	KF689642	KF689602	KF689612	KF689622	KF689632
P. panicorum	CM9s6	KF689651	KF689641	KF689601	KF689611	KF689621	KF689631
P. schizachyii J. Luo and N. Zhang	AL3s4	KF689650	KF689640	KF689600	KF689610	KF689620	KF689630
P. schizachyii	AL2m1	KF689649	KF689639	KF689599	KF689609	KF689619	KF689629
Pyricularia grisea Sacc.	M82	JX134670	JX134682	JX134656	JX134710	JX134724	JX134696
P. grisea	M83	JX134671	JX134683	JX134657	JX134711	JX134725	JX134697
P. oryzae Cavara	ATCC 4617	——————————Genome data, Broad Institute——————————					
P. oryzae	M25	JF414839	JF414888	JF414863	JF710397	JF710449	JF710422
P. oryzae	M60	JF414840	JF414889	JF414864	JF710398	JF710447	JF710423

[†]ATCC = American Type Culture Collection, Manassas, Virginia; CBS = Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands.

[‡]Species names in boldface indicate newly submitted sequences from this study.

Multilocus phylogenetic analyses and sequence data

There were 7,329 nucleotide characters included in the concatenated, six-gene dataset, of which 1,960 (26.7%) were parsimony informative (Table 4.1). Ambiguously aligned regions and gaps were eliminated prior to phylogenetic analyses. The general time reversible nucleotide substitution model with a discrete Gamma distribution rate variation across sites and a proportion of invariable sites (GTR + G + I) was selected and used for ML and BI analyses (Table B.2) (Tavare, 1986). BI and ML results were similar with respect to tree topology. ML bootstrap proportions ≥ 50% and BI posterior probabilities ≥ 0.95 are provided along with the ML tree (Fig 4.1).

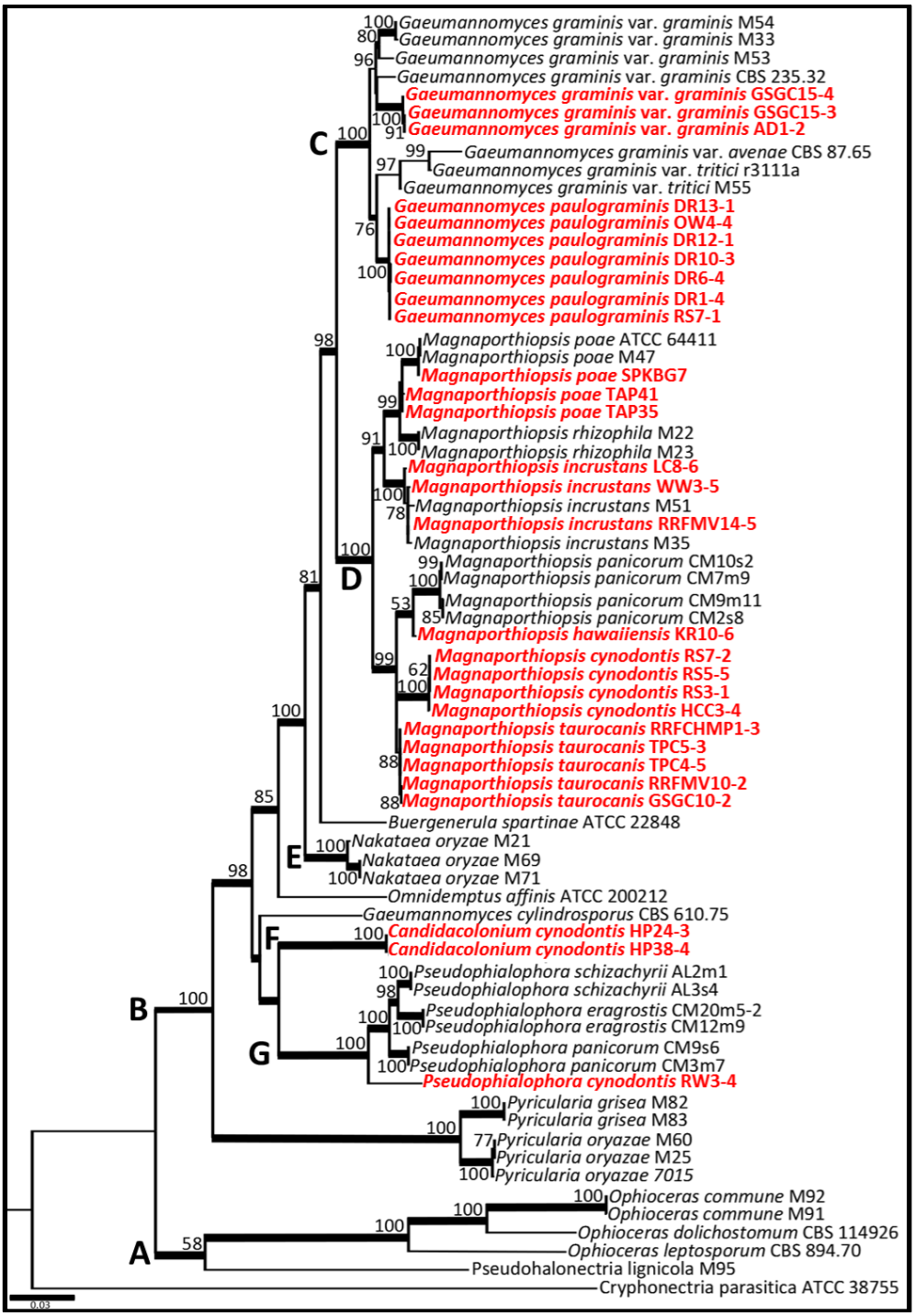

Figure 4.1 Maximum Likelihood phylogram based on the concatenated, six-gene (ITS, LSU, SSU, *MCM7*, *TEF1*, and *RPB1*) sequence dataset.

Boldfaced branches indicate BI posterior probabilities ≥ 0.95 and ML bootstrap values ≥ 50% are presented above internodes. Sequences of isolates displayed in red were generated from this study. All other data were obtained from public databases. *Cryphonectria parasitica* was chosen as the outgroup taxon.

The two analyses, BI and ML, separated all ingroup taxa into two main clades, A and B (Fig. 4.1). Clade A, which was supported by ML and BI analyses, included four species of *Ophioceras* and *Pseudohalonectria lignicola*. Clade B was highly supported and consisted of species belonging to *Gaeumannomyces*, *Magnaporthiopsis*, *Buergenerula*, *Nakataea*, *Omnidemptus*, *Candidacolonium*, *Pseudophialophora*, and *Pyricularia*.

Within clade B, three varieties of the type species of *Gaeumannomyces*, *G. graminis*, and a new species were grouped as subclade C. Four species of *Magnaporthiopsis* and three novel species constituted subclade D. Subclades E and F were comprised of three strains of *Nakataea oryzae* and two strains of a new species, respectively. Three species of *Pseudophialophora* and one novel species were grouped as subclade G. *Pyricularia grisea* and *P. oryzae* constituted subclade H.

Pairwise distance comparisons of species of *Gaeumannomyces* excluded *G. cylindrosporus,* as it was not situated within subclade C (Fig. 4.1). In consideration of the combined, six-gene dataset, interspecific pairwise distance comparisons ranged from 1.6 to 4.5%. The range in distance between isolate DR1–4, the type specimen of the new species, and other species of *Gaeumannomyces* was 1.9 to 4.1% with a mean of 2.5%. The mean interspecific distance within *Gaeumannomyces* was 2.7% (Fig. B.79).

Interspecific pairwise distance comparisons within *Magnaporthiopsis* averaged 2.4% with a range of 0.4 to 3.5%. Distances between type specimens of novel species and established species in the genus ranged from 0.9 to 3.5% in the case of isolate KR10–6, from 0.4 to 3.3% for isolate RS7–2, and from 0.4 to 3.2% for isolate RRFCHMP1–3.

Mean distances for isolates KR10–6, RS7–2, and RRFCHMP1–3 were 2.2, 2.1, and 2.2%, respectively (Fig. B.80).

Interspecific pairwise distance comparisons within *Pseudophialophora* ranged from 1.1 to 4.2%. The range in distance between isolate RW3–4, the type specimen of the novel species, and other species within *Pseudophialophora* was 4.1 to 4.2% with a mean of 4.1%. Mean interspecific distance for the genus was 2.5% (Fig. B.81).

Single-gene sequence data and phylogenetic analyses

Single gene datasets were aligned and analyzed to further assess the genetic affinities among fungal species in the study. These evaluations were conducted to determine topological concordance among genes used in the concatenated dataset and to determine which genes provide influence to genetic relations observed in the multilocus phylogeny.

Internal transcribed spacer region

Seven hundred eleven nucleotide characters were included in the ITS dataset, among which 251 (35.3%) were parsimony informative (Table 4.1). Ambiguously aligned regions and gaps were eliminated in phylogenetic analyses. The general time reversible nucleotide substitution model with a discrete Gamma distribution rate variation across sites (GTR + G) was selected and used for ML analyses (Table B.3) (Tavare, 1986). ML tree topology and ML bootstrap probabilities ≥ 75% are provided (Fig B.82). With exceptions for *Pseudohalonectria lignicola*, *G. cylindrosporus*, and *Omnidemptus affinis*, clades A and B were supported by the ML tree constructed with the ITS dataset.

Subclades C, D, E, F, G, and H were well supported by ML bootstrap values of 95, 99, 99, 99, 84, and 99%, respectively.

G. cylindrosporus was not included in pairwise distance comparisons of *Gaeumannomyces* spp. because of its distal location to the genus with respect to the multilocus phylogeny (Fig. 4.1). In consideration of the ITS dataset, interspecific pairwise distance comparisons ranged from 1.2 to 2.8% with a mean of 1.9%. The range in distance between isolate DR1–4, the type specimen of the novel species, and other species of *Gaeumannomyces* was 1.6 to 2.6% with a mean of 2.0% (Fig. B.83).

Interspecific pairwise distance comparisons within *Magnaporthiopsis* ranged from 0.2 to 5.7%. Differences between type specimens of novel species and established species in the genus ranged from 0.2 to 5.5% for isolate KR10–6, from 0.8 to 5.5% for isolate RS7–2, and from 0.4 to 5.3% for isolate RRFCHMP1–3. Mean differences between recognized species of *Magnaporthiopsis* and isolates KR10–6, RS7–2, and RRFCHMP1–3 were 3.0, 3.3, and 3.3%, respectively. Mean interspecific difference for the genus was 3.6% (Fig. B.84).

Interspecific pairwise distance comparisons within *Pseudophialophora* ranged from 2.5 to 7.7%. The range in difference between the type specimen of the novel species, isolate RW3–4, and other species within *Pseudophialophora* was 6.8 to 7.7% with a mean of 7.2%. Mean interspecific difference for the genus was 4.8% (Fig. B.85).

Large subunit gene

One hundred forty-three (15.1%) of the 946 nucleotide characters included in the LSU dataset were parsimony informative (Table 4.1). Gaps and ambiguously aligned regions were excluded prior to phylogenetic analyses. The Kimura 2-parameter

nucleotide substitution model with a discrete Gamma distribution rate variation across sites and a proportion of invariable sites (K2 + G + I) was selected and used for ML analyses (Table B.4) (Kimura, 1980). ML bootstrap proportions ≥ 75% and ML tree topology are provided (Fig. B.86). With an exception for *Pseudohalonectria lignicola*, the ML tree constructed with the LSU dataset provided ML bootstrap support values of 100 and 99% for clades A and B, respectively. Subclades C, D, E, F, G, and H were also well supported by ML bootstrap values of 94, 85, 100, 100, 99, and 100%, respectively.

G. cylindrosporus was not included in pairwise distance comparisons of *Gaeumannomyces* spp. because of its distal location to the genus with respect to the multilocus phylogeny (Fig. 4.1). Interspecific pairwise distance comparisons ranged from 0.2 to 0.6%. The range in distance between isolate DR1–4, the type specimen of the novel species, and other species of *Gaeumannomyces* was 0.4 to 0.6% with a mean of 0.4%. The mean interspecific distance within *Gaeumannomyces* was 0.4% (Fig. B.87).

Interspecific pairwise distance comparisons within *Magnaporthiopsis* ranged from 0.1 to 1.9%. Differences between type specimens of novel species and established species in the genus ranged from 0.1 to 1.8% for isolate KR10–6, from 0.2 to 1.8% for isolate RS7–2, and from 0.1 to 1.9% for isolate RRFCHMP1–3. Mean differences between recognized species of *Magnaporthiopsis* and isolates KR10–6, RS7–2, and RRFCHMP1–3 were 1.1, 1.2, and 1.4%, respectively. Mean interspecific difference for the genus was 1.3% (Fig. B.88).

Interspecific pairwise distance comparisons within *Pseudophialophora* ranged from 0.4 to 1.2%. The range in difference between the type specimen of the novel

species, isolate RW3–4, and other species within *Pseudophialophora* was 0.8 to 1.2% with a mean of 1.0%. Mean interspecific difference for the genus was 0.8% (Fig. B.89).

Small subunit gene

There were 1,112 nucleotide characters included in the SSU dataset, of which 415 (37.3%) were parsimony informative (Table 4.1). Ambiguously aligned regions and gaps were eliminated prior to phylogenetic analyses. The Kimura 2-parameter nucleotide substitution model with a discrete Gamma distribution rate variation across sites (K2 + G) was selected and used for ML analyses (Table B.5) (Kimura, 1980). ML tree topology and ML bootstrap probabilities ≥ 75% are provided (Fig. B.90). The ML tree constructed with the SSU dataset did not provide adequate resolutions for relationships among ingroup taxa. However, the single-gene phylogeny did provide marginal support for groupings of clades A and B.

Pairwise distance comparisons of species of *Gaeumannomyces* excluded *G. cylindrosporus,* as it was not situated within subclade C in the multilocus ML phylogeny (Fig. 4.1). In consideration of the SSU dataset, interspecific pairwise distance comparisons ranged from 0.0 to 4.8% with a mean of 0.9%. The range in distance between isolate DR1–4, the type specimen of the novel species, and other species of *Gaeumannomyces* was 0.0 to 4.6% with a mean of 0.5% (Fig. B.91).

Interspecific pairwise distance comparisons within *Magnaporthiopsis* ranged from 0.0 to 4.8%. Differences between type specimens of novel species and established species in the genus ranged from 0.4 to 4.6% for isolate KR10–6, from 0.2 to 4.8% for isolate RS7–2, and from 0.2 to 4.6 for isolate RRFCHMP1–3. Mean differences between recognized *Magnaporthiopsis* species and isolates KR10–6, RS7–2, and RRFCHMP1–3

were 3.2, 1.9, and 2.0%, respectively. Mean interspecific difference for the genus was 2.4% (Fig. B.92).

Interspecific pairwise distance comparisons within *Pseudophialophora* ranged from 0.0 to 0.4%. The range in difference between the type specimen of the novel species, isolate RW3–4, and other species within *Pseudophialophora* was 0.2 to 0.4% with a mean of 0.2%. Mean interspecific difference for the genus was 0.1% (Fig. B.93).

Minichromosome maintenance complex component 7 gene

Six hundred twenty-five nucleotide characters were included in the *MCM7* dataset, among which 245 (39.2%) were parsimony informative (Table 4.1). Prior to phylogenetic analyses, gaps and ambiguously aligned regions were eliminated. The Tamura 3-parameter nucleotide substitution model with a discrete Gamma distribution rate variation across sites (T92 + G) was selected and used for ML analyses (Table B.6) (Tamura, 1992). ML bootstrap proportions ≥ 75% are provided along with ML tree topology (Fig. B.94). With an exception for *Pseudohalonectria lignicola*, the ML tree constructed with the *MCM7* dataset supported the multilocus ML phylogeny. ML bootstrap support values were 99, 83, 96, 100, 96, and 100% for clades A, D, E, F, G, and H, respectively.

G. cylindrosporus was not included in pairwise distance comparisons of species of *Gaeumannomyces* because of its distal location to the genus with respect to the multilocus phylogeny (Fig. 4.1). Interspecific pairwise distance comparisons ranged from 2.2 to 6.7%. The range in distance between isolate DR1–4, the type specimen of the novel species, and other species of *Gaeumannomyces* was 4.1 to 6.5% with a mean of 5.0%. The mean interspecific distance within *Gaeumannomyces* was 5.0% (Fig. B.95).

Interspecific pairwise distance comparisons within *Magnaporthiopsis* ranged from 0.0 to 7.5%. Differences between type specimens of novel species and established species in the genus ranged from 0.0 to 6.5% for isolate KR10–6, from 0.0 to 6.5% for isolate RS7–2, and from 0.0 to 6.5% for isolate RRFCHMP1–3. Mean differences between recognized species of *Magnaporthiopsis* and isolates KR10–6, RS7–2, and RRFCHMP1–3 were 2.9, 3.1, and 3.4%, respectively. Mean interspecific difference for the genus was 6.0% (Fig. B.96).

Interspecific pairwise distance comparisons within *Pseudophialophora* ranged from 1.5 to 5.6%. The range in difference between the type specimen of the novel species, isolate RW3–4, and other species within *Pseudophialophora* was 4.9 to 5.6% with a mean of 5.3%. Mean interspecific difference for the genus was 3.1% (Fig. B.97).

Translation elongation factor 1-alpha gene

Two hundred sixty-five (27.0%) of the 983 nucleotide characters included in the *TEF1* dataset were parsimony informative (Table 4.1). Ambiguously aligned regions and gaps were eliminated in phylogenetic analyses. The Tamura-Nei nucleotide substitution model with a discrete Gamma distribution rate variation across sites (TN93 + G) was selected and used for ML analyses (Table B.7) (Tamura and Nei, 1993). ML bootstrap proportions ≥ 75% are provided (Fig. B.98). Clades A and B were supported by the ML tree constructed with the *TEF1* dataset. Minor discrepancies existed among groupings of subclades C and D, compared to the combined six-gene ML tree topology; however, subclades E, F, G, and H were well supported by bootstrap proportions of 98, 100, 98, and 100%, respectively.

G. cylindrosporus was not included in pairwise distance comparisons of *Gaeumannomyces* spp. because of its distal location to the genus with respect to the multilocus phylogeny (Fig. 4.1). In consideration of the *TEF1* dataset, interspecific pairwise distance comparisons ranged from 1.1 to 4.9% with a mean of 3.4%. The range in distance between isolate DR1–4, the type specimen of the novel species, and other species of *Gaeumannomyces* was 2.4 to 4.4% with a mean of 3.2% (Fig. B.99).

Interspecific pairwise distance comparisons within *Magnaporthiopsis* averaged 1.4% with a range of 0.5 to 2.6%. Distances between type specimens of novel species and established species in the genus ranged from 1.2 to 2.6% in the case of isolate KR10–6, from 0.5 to 2.3% for isolate RS7–2, and from 0.5 to 2.4% for isolate RRFCHMP1–3. Mean distances for isolates KR10–6, RS7–2, and RRFCHMP1–3 were 1.9, 1.6, and 1.6%, respectively (Fig. B.100).

Interspecific pairwise distance comparisons within *Pseudophialophora* ranged from 0.9 to 4.8%. The range in difference between the type specimen of the novel species, isolate RW3–4, and other species within *Pseudophialophora* was 4.5 to 4.8% with a mean of 4.7%. Mean interspecific difference for the genus was 2.8% (Fig. B.101).

RNA polymerase II gene

There were 836 nucleotide characters included in the *RPB1* dataset, of which 431 (51.6%) were parsimony informative (Table 4.1). Gaps and ambiguously aligned regions were excluded in phylogenetic analyses. The Tamura-Nei nucleotide substitution model with a discrete Gamma distribution rate variation across sites and a proportion of invariable sites (TN93 + G + I) was selected and used for ML analyses (Table B.8) (Tamura and Nei, 1993). ML bootstrap proportions ≥ 75% are provided (Fig. B.102).

The ML tree constructed with the *RPB1* dataset provided ML bootstrap support values of 97 and 99% for clades A and B, respectively. Subclades C, D, E, F, G, and H were also well supported, each having ML bootstrap proportions of 100%.

G. cylindrosporus was not included in pairwise distance comparisons of *Gaeumannomyces* spp. because of its distal location to the genus with respect to the multilocus phylogeny (Fig. 4.1). Interspecific pairwise distance comparisons ranged from 0.0 to 8.9% with a mean of 5.2%. The range in distance between isolate DR1–4, the type specimen of the novel species, and other species of *Gaeumannomyces* was 0.0 to 7.8% with a mean of 4.6% (Fig. B.103).

Interspecific pairwise distance comparisons within *Magnaporthiopsis* ranged from 0.2 to 4.2%. Differences between type specimens of novel species and established species in the genus ranged from 0.2 to 3.8% for isolate KR10–6, from 0.3 to 4.2% for isolate RS7–2, and from 0.2 to 4.0% for isolate RRFCHMP1–3. Mean differences between recognized *Magnaporthiopsis* species and isolates KR10–6, RS7–2, and RRFCHMP1–3 were 1.7, 2.1, and 2.1%, respectively. Mean interspecific difference for the genus was 2.3% (Fig. B.104).

Interspecific pairwise distance comparisons within *Pseudophialophora* ranged from 1.2 to 7.4%. The range in difference between the type specimen of the novel species, isolate RW3–4, and other species within *Pseudophialophora* was 7.0 to 7.4% with a mean of 7.3%. Mean interspecific difference for the genus was 3.8% (Fig. B.105).

Optimum temperature for *in vitro* growth

In general, isolates grew most aggressively at temperatures of 25 and 32 C and least aggressively at temperatures of 18 and 38 C (Figs. B.106 – B.134). Mean optimal growing temperatures for *C. cynodontis*, *G. graminis* var. *graminis*, *G. paulograminis*, *M. cynodontis*, *M. hawaiiensis*, *M. incrustans*, *M. poae*, *M. taurocanis*, and *P. cynodontis* were 27.8, 24.5, 27.1, 27.1, 26.9, 26.4, 27.0, 27.5, and 30.6 C, respectively (Table 4.3).

Table 4.3 Species name, Isolate ID, and optimal temperatures for mycelial growth of ectotrophic root-infecting fungal specimens grown seven days on potato dextrose agar.

Fungal species	Isolate ID	Optimal temperature (C)
C. cynodontis	HP24–3	27.9
	HP38–4	27.7
G. graminis var. *graminis*	AD1–2	24.8
	GSGC15–3	23.9
	GSGC15–4	24.7
G. paulograminis	DR1–4	26.9
	DR6–4	26.9
	DR10–3	27.2
	DR12–1	27.1
	DR13–1	27.3
	OW4–4	26.9
	RS7–1	27.4
M. cynodontis	HCC3–4	27.3
	RS3–1	27.0
	RS5–5	26.9
	RS7–2	27.0
M. hawaiiensis	KR10–6	26.9
M. incrustans	LC8–6	25.3
	RRFMV14–5	26.6
	WW3–5	27.2
M. poae	SPKBG7	27.0
	TAP35	26.4
	TAP41	27.5
M. taurocanis	GSGC10–2	27.3
	RRFCHMP1–3	28.9
	RRFMV10–2	27.1
	TPC4–5	27.3
	TPC5–3	27.1
P. cynodontis	RW3–4	30.6

Taxonomy

In consideration of morphological and biological characteristics in conjunction with molecular phylogenetic studies, a distinct monophyletic genus with one novel species, one novel species of *Gaeumannomyces*, three novel species of *Magnaporthiopsis*, and one novel species of *Pseudophialophora* are described. Cultures of type specimens were dried for storage at the U.S. National Fungus Collections Herbarium (Fig. B.135)

***Candidacolonium* P. L. Vines and M. Tomaso-Peterson, gen. nov.**

MycoBank: 812290

Etymology: The generic name refers to the luminous white colony characteristics for the axenic culture of the generic type specimen.

Type species: Candidacolonium cynodontis.

Habit: On roots of Poaceae plants.

Known distribution: Texas, USA.

Notes: The two collections included shared many characteristics and formed a monophyletic clade. The novel genus, *Candidacolonium*, is typified by *C. cynodontis*, which, in conjunction with *G. graminis* var. *graminis* and *G. paulograminis*, are polyphyletic in their formation of hyphopodia. *G. graminis var. graminis* and *G. paulograminis* produce deeply lobed and crenately lobed hyphopodia, respectively. These hyphophodial shapes are distinguishable from the chiroid or mitten-shaped hyphopodia of *C. cynodontis*.

***Candidacolonium cynodontis* P. L. Vines and M. Tomaso-Peterson, sp. nov.**

MycoBank: 812291

BPI: 893105

Etymology: The specific epithet refers to the host's generic name.

Specimens examined: United States, Texas: Houston, 29°45'37.5"N, 95°22'11.3"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson HP24–3.
United States, Texas: Houston, 29°45'37.5"N, 95°22'11.3"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson HP38–4.

Notes: Colony diameter was approximately 19.0 mm on PDA in the dark after 7 days at 25 C. Colony color was bright white at 7-day maturity, and pale to dark grayish buff at 14- to 21-day maturity. Colony was reverse pigmented pale to dark grayish buff. Hyphae were septate, (2.5-) 2.4-3.2 (-3.8) µm (mean = 2.8; S.D. = 0.4; n = 25) wide, and hyaline to brown in color. Hyaline to brown colored stigmatopods measured (5.0-) 6.6-10.6 (-11.3) × (5.0-) 4.6-7.0 (-7.5) µm (mean = 5.8 × 8.6; S.D. = 1.2, 2.0; n = 25) and gave rise to brown to olivaceous brown, chiroid or mitten-shaped hyphopodia that measured (10.0-) 10.2-13.0 (-15.0) × (7.5-) 7.2-10.4 (-12.5) µm (mean = 11.6 × 8.8; S.D. = 1.4, 1.6; n = 25).

***Gaeumannomyces paulograminis* P. L. Vines and M. Tomaso-Peterson, sp. nov.**

MycoBank: 812292

BPI: 893101

Etymology: The specific epithet is a Latin portmanteau of the English words "little" and "grass" to describe host characteristics.

Specimens examined: United States, Mississippi: Choctaw, 32°49'09"N, 89°07'52.3"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson *DR1–4*.
United States, Mississippi: Choctaw, 32°49'09"N, 89°07'52.3"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson *DR6–4*.
United States, Mississippi: Choctaw, 32°49'09"N, 89°07'52.3"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson *DR10–3*.
United States, Mississippi: Choctaw, 32°49'09"N, 89°07'52.3"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson *DR12–1*.
United States, Mississippi: Choctaw, 32°49'09"N, 89°07'52.3"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson *DR13–1*.
United States, Mississippi: West Point, 33°36'26.4"N, 88°39'06.2"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson *OW4–4*.
United States, Texas: Humble, 29°59'54"N, 95°15'43.8"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson *RS7–1*.

Notes: Colony diameter was approximately 35.7 mm on PDA in the dark after 7 days at 25 C. Colony color was dark olivaceous gray and reverse pigmented pale greenish black. Hyphae were septate, (2.5-) 3.1-5.5 (-5.6) µm (mean = 4.3; S.D. = 1.2; n = 25) wide, hyaline to brown in color, and produced brown to olivaceous brown, crenately lobed hyphopodia that measured (20.0-) 21.5-28.7 (-32.5) × (17.5-) 18.6-24.4 (-30.0) µm (mean = 25.1 × 21.5; S.D. = 3.6, 2.9; n = 25).

***Magnaporthiopsis hawaiiensis* P. L. Vines and M. Tomaso-Peterson, sp. nov.**

MycoBank: 812293

BPI: 893104

Etymology: The specific epithet refers to the location of collection, Hawaii.

Specimens examined: United States, Hawaii: Maui, 20°47'54.1"N, 156°19'54.9"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson *KR10–6*.

Notes: Colony diameter was approximately 27.7 mm on PDA in the dark after 7 days at 25 C. Colonies were hyaline with dark, umber brown concentric zones, and reverse pigmented the same colors. Hyphae were septate, (3.8-) 3.8-5.0 (-5.6) µm (mean = 4.4; S.D. = 0.6; n = 25) wide, hyaline to brown in color.

***Magnaporthiopsis cynodontis* P. L. Vines and M. Tomaso-Peterson, sp. nov.**

MycoBank: 812294

BPI: 893103

Etymology: The specific epithet refers to the host's generic name.

Specimens examined: United States, Texas: Houston, 29°45'35"N, 95°29'21.1"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson *HCC3–4*.
United States, Texas: Humble, 29°59'54"N, 95°15'43.8"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson *RS3–1*.
United States, Texas: Humble, 29°59'54"N, 95°15'43.8"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson *RS5–5*.
United States, Texas: Humble, 29°59'54"N, 95°15'43.8"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson *RS7–2*.

Notes: Colony diameter was approximately 34.5 mm on PDA in the dark after 7 days at 25 C. Colony color was dark greenish gray and reverse pigmented pale greenish black. Hyphae were septate, (2.5-) 3.1-4.9 (-5.0) µm (mean = 4.0; S.D. = 0.9; n = 25) wide, hyaline to brown in color.

***Magnaporthiopsis taurocanis* P. L. Vines and M. Tomaso-Peterson, sp. nov.**

MycoBank: 812295

BPI: 893100

Etymology: The type specimen was collected in Starkville, Mississippi, home to Mississippi State University. The specific epithet is a Latin portmanteau of the English words "bull" and "dog" in honor of Mississippi State University's English Bulldog mascot.

Specimens examined: United States, Alabama: Birmingham, 33°25'46.7"N, 86°39'05.4"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson *GSGC10–2*.
United States, Mississippi: Starkville, 33°27'01.4"N, 88°49'06.2"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson *RRFCHMP1–3*.
United States, Mississippi: Starkville, 33°27'01.4"N, 88°49'06.2"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson *RRFMV10–2*.
United States, Tennessee: Memphis, 35°04'09.1"N, 89°52'01.4"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson *TPC4–5*.
United States, Tennessee: Memphis, 35°04'09.1"N, 89°52'01.4"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson *TPC5–3*.

Notes: Colony diameter was approximately 30.5 mm on PDA in the dark after 7 days at 25 C. Colonies were hyaline with dark, umber brown zones, and reverse pigmented the same colors. Hyphae were septate, (2.5-) 2.9-4.9 (-5.0) µm (mean = 3.9; S.D. = 1.0; n = 25) wide, hyaline to brown in color.

***Pseudophialophora cynodontis* P. L. Vines and M. Tomaso-Peterson, sp. nov.**

MycoBank: 812296

BPI: 893102

Etymology: The specific epithet refers to the host's generic name.

Specimens examined: United States, Tennessee: Memphis, 35°08'58.3"N, 90°02'56.3"W. Roots of *Cynodon dactylon* × *C. transvaalensis*, P. L. Vines and M. Tomaso-Peterson *RW3–4*.

Notes: Colony diameter was approximately 7.75 mm on PDA in the dark after 7 days at 25 C. Colony color was grayish pale yellow green and reverse pigmented pale yellowish gray. Hyphae were septate, (2.5-) 2.4-3.0 (-3.1) µm (mean = 2.7; S.D. = 0.3; n = 25) wide, hyaline to brown in color.

Discussion

In accordance with previous studies, these results disclose a distinct divergence between saprophytic and parasitic taxa in Magnaporthaceae (Cannon, 1994; Zhang et al., 2011; Luo and Zhang, 2013; Luo et al., 2014). Topologies of the concatenated dataset and individual gene datasets were highly homologous, and single-gene analyses provided great support for the six-gene, concatenated phylogeny. Clade A, constituted by members of *Ophioceras* and *Pseudohalonectria*, includes saprophytic species that are commonly observed on submerged woody substrates (Luo et al., 2014). A grouping of plant parasitic species in the genera *Gaeumannomyces*, *Magnaporthiopsis*, *Buergenerula*, *Nakataea*, *Omnidemptus*, *Candidacolonium*, *Pseudophialophora*, and *Pyricularia* formed the monophyletic clade B, which comprises two distinct lineages. The earliest, clade H, is established by the gray leaf spot fungus, *Pyricularia grisea*, and the rice blast fungus, *Pyricularia oryzae*, both of which produce leaf-infecting sympodial conidia (Luo et al., 2014). The second lineage is primarily composed of fungi associated with roots of

Poaceae. Apart from *Nakatae oryzae*, which produces sympodial conidia, fungi in the second lineage typically have *Phialophora*- or *Harpophora*-like asexual states (Cannon, 1994; Zhang et al., 2011; Luo and Zhang, 2013). The novel species proposed in this study belong in the second lineage of clade B. This concept is supported by a six-gene phylogenetic analysis, morphological and biological characteristics, and grass root associated habit of the fungi.

Prior to this study, the only recognized turfgrass root pathogens in Magnaporthaceae that exhibit ectotrophic growth habits were *G. graminis* var. *graminis*, *G. graminis* var. *avenae*, and *M. poae*. Of the three, only *G. graminis* var. *graminis* is associated with *Cynodon* spp.; *G. graminis* var. *avenae* and *M. poae* are most commonly associated with cool-season grasses such as *Agrostis*, *Festuca*, and *Poa* spp. *M. incrustans* has been isolated from bermudagrass roots, but its role in disease incitation has not been confirmed (Elliott, 1991). As suspected, based on previous research efforts, *G. graminis* var. *graminis* and *M. incrustans* were isolated and identified from roots of ultradwarf bermudagrasses in this study. Other ERI fungal species associated with *Cynodon* spp. include the causal agents of spring dead spot, namely, *Ophiosphaerella herpotricha*, *O. korrae*, and *O. narmari*. *Ophiosphaerella* spp. are members of the Phaeosphaeriaceae, which is a closely-related family to the Magnaporthaceae, from which the fungi in this study are associated. *Ophiosphaerella* spp. were not isolated from roots of ultradwarf bermudagrasses in this study.

In addition to identification of recognized ERI fungal species, phylogenetic analyses of individual and combined gene datasets disclosed the presence and identity of six novel ERI fungal species associated with roots of ultradwarf bermudagrasses affected

by summer decline. Seven isolates formed a distinct, monophyletic clade within *Gaeumannomyces*, and were identified as *G. paulograminis* sp. nov. *G. graminis* var. *graminis* and *G. paulograminis* are morphologically distinguishable by hyphopodial characteristics. *G. paulograminis* has crenately lobed hyphopodia, whereas hyphopodia of *G. graminis* var. *graminis* are deeply lobed.

Three new species, *M. hawaiiensis*, *M. cynodontis*, and *M. taurocanis*, represented by one, four, and five isolates, respectively, were grouped within *Magnaporthiopsis*. *M. hawaiiensis*, *M. cynodontis*, and *M. taurocanis* consistently diverged from other species of *Magnaporthiopsis*, forming three distinct lineages most closely related to *M. panicorum*. *Magnaporthiopsis* spp. discussed here are distinguished from other ERI fungal species in this study by having appressed mycelium, contrasted to aerial or cottony mycelium as observed among the others.

Two ERI fungal isolates erected a well-supported, monophyletic clade and were identified as *C. cynodontis* sp. nov. gen. nov. *C. cynodontis*, along with *G. graminis* var. *graminis* and *G. paulograminis* are polyphyletic with respect to their formation of hyphopodia. *C. cynodontis* produces chiroid or mitten-shaped hyphopodia, which are contrasted to deeply lobed and crenately lobed hyphopodia of *G. graminis* var. *graminis* and *G. paulograminis*, respectively.

Lastly, a single isolate was consistently grouped within *Pseudophialophora* and identified as *P. cynodontis*. This genus was recently described by Luo et al. (2014) as a collection of six specimens, representing three species. Namely, *P. eragrostis*, *P. panicorum*, and *P. schizachyrii*, the three species were collected from healthy grass roots and did not cause disease symptoms on the hosts (Luo et al., 2014). The close relation of

P. cynodontis to the non-pathogenic species of *Pseudophialophora* may provide reasoning for the decreased isolation frequency, compared to other ERI fungal species in the study.

In conclusion, six novel ERI fungi and two recognized ERI fungi, all in the Magnaporthaceae, were identified from roots of ultradwarf bermudagrasses that were symptomatic for summer decline. Prior to this study, *G. graminis* var. *graminis* and *M. incrustans* were the only known ERI fungi in the Magnaporthaceae associated with *Cynodon* spp. These findings bring about inquiries regarding the role each of these fungal species plays in the decline of ultradwarf bermudagrass putting greens in the late summer and early fall months in the Deep South. Pathogenicity of the recognized ERI fungal species to bermudagrass has been studied previously (Elliott, 1991). In that study, *G. graminis* var. *graminis* was pathogenic to bermudagrass, whereas *M. incrustans* was not (Elliott, 1991). Answers to questions regarding effects of ERI fungi on ultradwarf bermudagrass will be of utmost importance to those who manage these grasses daily, the golf course superintendents.

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CHAPTER V

PATHOGENICITY AND ORIGIN OF ECTOTROPHIC ROOT-INFECTING FUNGI ISOLATED FROM ROOTS OF ULTRADWARF BERMUDAGRASS

Abstract

Roots of ultradwarf bermudagrasses affected by summer decline are typically brown to black in color, diminutive in size, and frequently colonized with dark runner hyphae, growth cessation structures, and simple and lobed hyphopodia, which are characteristic signs of ectotrophic root-infecting (ERI) fungi. Research at Mississippi State University (MSU) led to the identification of six novel fungal species (*Candidacolonium cynodontis, Gaeumannomyces paulograminis*, *M. cynodontis, Magnaporthiopsis hawaiiensis*, *M. taurocanis*, and *Pseudophialophora cynodontis*) and two recognized species (*G. graminis* var. *graminis* and *M. incrustans*) from infected roots. The objectives of this study were to screen ERI fungal species for pathogenicity on 'Champion' and 'MiniVerde' ultradwarf bermudagrasses, and summarize the origins of each fungal species. *In vivo* inoculations of ERI fungi were successful in recreating symptomatology associated with summer decline on ultradwarf bermudagrass; however, various levels of pathogenicity among ERI fungal species were observed. *C. cynodontis and G. paulograminis* were most aggressive and incited greatest root disease of all fungal species evaluated. Assessments of colony morphology demonstrated a widespread origin of ERI fungi across the southern U.S. In numerous instances, multiple fungal species

were isolated from a single turfgrass sample, suggesting a cohabitant nature among ERI fungi.

Introduction

Summer decline has been observed throughout the Deep South United States on hybrid bermudagrass (*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy) managed as golf course putting greens. This disease occurs most readily during late summer and early fall months, when turfgrass plants experience augmented environmental and mechanical stresses such as extreme temperatures, increased foot traffic, and reduced mowing heights. Symptoms of summer decline have been described previously (Chapter IV).

Two recognized fungal species, *Gaeumannomyces graminis* (Sacc.) Arx and D. Olivier var. *graminis* and *Magnaporthiopsis incrustans* (Landschoot and Jackson) J. Luo and N. Zhang (≡ *G. incrustans* Landschoot and Jackson), and six novel species, *Candidacolonium cynodontis* P. L. Vines and M. Tomaso-Peterson, *G. paulograminis* P. L. Vines and M. Tomaso-Peterson, *M. cynodontis* P. L. Vines and M. Tomaso-Peterson, *M. hawaiiensis* P. L. Vines and M. Tomaso-Peterson, *M. taurocanis* P. L. Vines and M. Tomaso-Peterson, and *Pseudophialophora cynodontis* P. L. Vines and M. Tomaso-Peterson, were isolated and identified from ultradwarf bermudagrass roots displaying symptoms of summer decline (Chapter IV).

Generally, *G. graminis* var. *graminis* is not considered a serious pathogen of Poaceae; however, pathogenicity of *G. graminis* var. *graminis* to hybrid bermudagrass has been demonstrated and it is considered the causal agent for bermudagrass decline (Deacon, 1981; Elliott, 1991). In previous evaluations, *M. incrustans* was reported to

express weak virulence to annual bluegrass (*Poa annua* L.) and Kentucky bluegrass (*Poa pratensis* L.) and did not affect hybrid bermudagrass (Landschoot and Jackson, 1990; Elliott, 1991).

The objective of this research was to evaluate novel and recognized ERI fungal species as possible incitants for summer decline of ultradwarf bermudagrass.

Materials and methods

With the exception of growing fungal inoculum, which was conducted at the MSU-Turfgrass Pathology Laboratory in Dorman Hall Room 219, Mississippi State, MS, all inoculation procedures were conducted at Rodney R. Foil Research Center (RRFRC) in Starkville, MS. Three studies, outlined in detail in following sections, were initiated 18, 19, and 20 Aug 2014 and terminated 13, 14, and 15 Oct 2014, respectively.

Isolation, identification, and selection of fungal strains

Fungal isolation and identification was conducted as defined in Chapter IV. Eight species, two previously recognized and six newly described, were identified from roots of ultradwarf bermudagrasses colonized with dark, runner hyphae. Representative specimens (Fig. 5.1) were selected for use in pathogenicity evaluations. Fungi were plated on PDA (39 g liter^{-1}) and incubated at 25 C for approximately 14 days prior to inoculation.

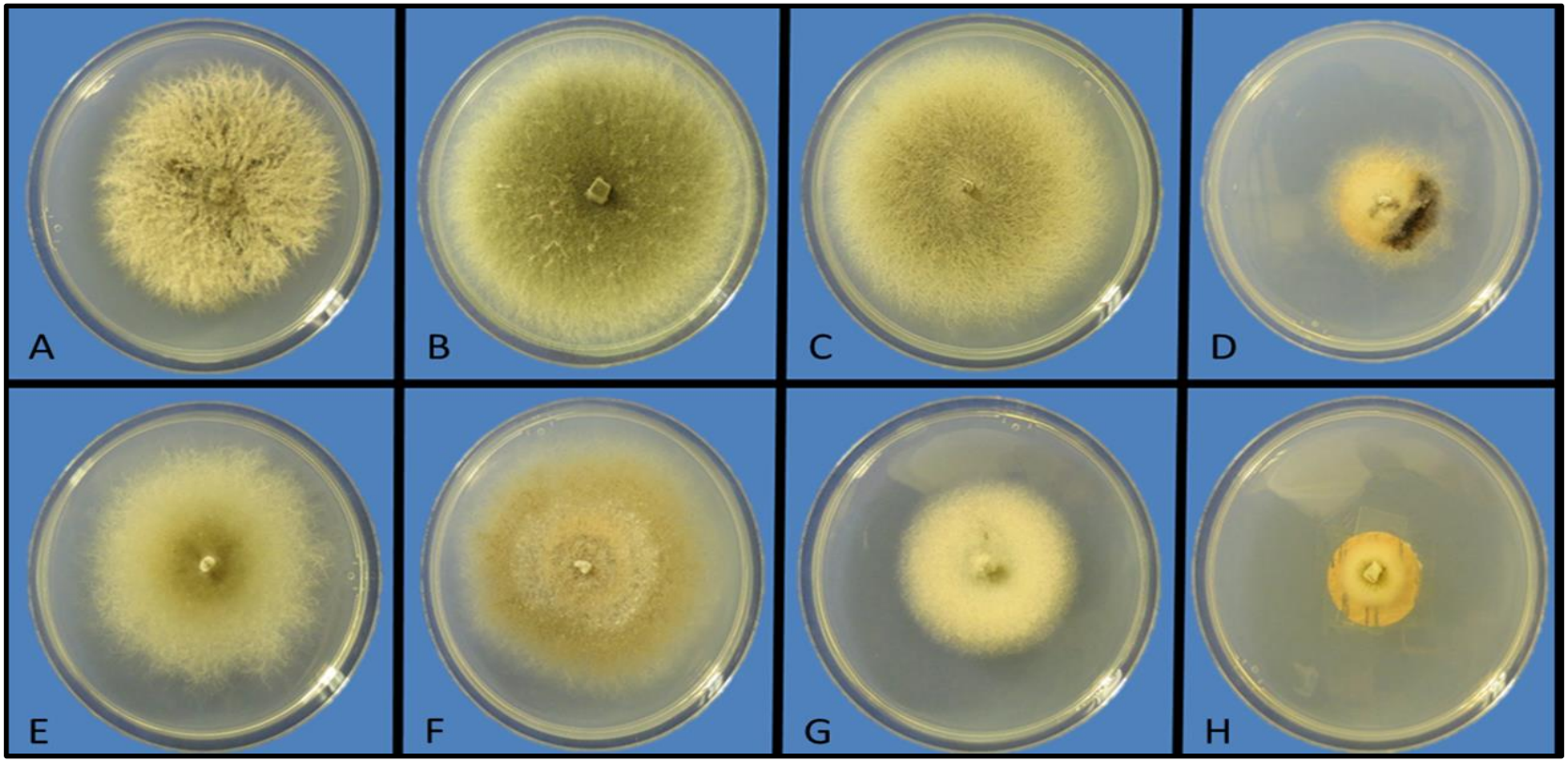

Figure 5.1 Colony morphology of representative ectotrophic root-infecting fungal isolates at 7-day maturity on PDA.

A = *Gaeumannomyces graminis* var. *graminis*; B = *G. paulograminis*, C = *Magnaporthiopsis incrustans*, D = *M. hawaiiensis*, E = *M. cynodontis*, F = *M. taurocanis*, G = *Candidacolonium cynodontis*, H = *Pseudophialophora cynodontis*.

Origins of fungal species

Golf course superintendents observing summer decline symptoms on ultradwarf bermudagrass putting greens submitted samples (10.8 cm diameter) to the MSU-Plant Disease Diagnostic Laboratory. Roots of affected plants were examined with a stereomicroscope for presence of runner hyphae, lobed and simple hyphopodia, and growth cessation structures. Colonized roots were subjected to fungal isolation as outlined in Chapter IV, and pure cultures were attained. Axenic fungal cultures were identified at maturation levels of 7 and 10 days based on relatedness to colony morphologies of representative isolates (Fig. 5.1). Additionally, documentation was made on host cultivar from which ERI fungi were isolated.

Plant material

Samples (10.8 cm diameter) of 'Champion' and 'MiniVerde' ultradwarf bermudagrass were collected from research plots at RRFRC and positioned in plastic pots (Fig. C.1). Potted grasses were placed in a greenhouse, irrigated to maintain adequate soil moisture, fertilized every 14 days with a 24N–8P_2O_5–16K_2O Miracle-Gro fertilizer (Scotts Company, Marysville, OH, USA) and allowed to produce an abundance of aerial stoloniferous material (Fig. C.2). Stolons were gathered and cut into pieces that consisted of five nodes and four internodes (Fig. C.3).

Inoculation

Methods for inoculation used in this study were adopted and modified from previous research (Wong et al., 2012). Premium Play Sand® (The QUIKRETE® Companies, Atlanta, GA, USA) was sterilized (Fig. C.4) and placed into inoculation

containers made of 7.6 cm diameter polyvinyl chloride (Fig. C.5). PDA, fully-colonized with a given fungal isolate, was cut into 1 cm^2 sections and placed atop sand to serve as fungal inoculum (Fig. C.6). Five grass plants, each containing five nodes and four internodes, were placed in direct contact with fungal inoculum (Fig. C.7), pots were capped with approximately 1 cm of sterilized sand for moisture retention (Fig. C.8), and placed in temperature- and light-controlled growing chambers for 8 weeks (Fig C.9). Growth chamber photoperiods were adjusted to 12 hours and soil temperatures were maintained near 30 C, providing optimal growing conditions for fungal inoculum (Chapter IV). Plants were watered to maintain adequate soil moisture and fertilized every 14 days as previously described to promote growth.

Environmental conditions were monitored throughout the study and summarized in Table 5.1. Soil temperatures were measured by placing a WatchDog B-Series Button Logger (Spectrum Technologies, Aurora, IL, USA) into the soil layer of a container in each of the three growth chambers (Fig. C.10). Soil temperature measurements were recorded hourly throughout the study. Photosynthetically active radiation (PAR) light values were recorded with Lightscout Quantum Light Sensors (Spectrum Technologies, Aurora, IL, USA) and WatchDog 1400 data loggers (Spectrum Technologies, Aurora, IL, USA) (Fig. C.11). Measurements of air temperature, relative humidity, and dew point were taken with an EL-USB-2-LCD temperature and humidity data logger (Lascar Electronics, Inc., Erie, PA, USA) hourly for the duration of the study (Fig. C.12).

Table 5.1 Environmental conditions of growth chambers used in pathogenicity studies.

Daytime	GC[†]1	GC2	GC3	Mean[‡]
Air Temperature (C)	33.96	33.55	33.38	33.63
Soil Temperature (C)	29.51	30.48	29.89	29.96
Relative Humidity (%)	70.72	71.29	63.67	68.56
Dew Point (C)	27.83	27.57	25.50	26.97
PAR[§] (μmol m^{-2} sec^{-1})	274.60	240.95	245.75	253.77
Nighttime				
Air Temperature (C)	27.37	26.48	30.35	28.07
Soil Temperature (C)	26.94	27.22	30.64	28.27
Relative Humidity (%)	92.88	93.43	92.79	93.03
Dew Point (C)	26.10	25.32	29.03	26.82

[†] Growth Chamber.
[‡] Mean, expressed as the average of GC1, GC2, and GC3.
[§] Photosynthetically Active Radiation, measured via LightScout Quantum Light Sensor.

Design and analysis of experiment

Studies were arranged in a randomized complete block design with a split-plot constraint. Ultradwarf bermudagrass cultivars were whole-plot factors and fungal species were split-plot factors. There were 20 treatment combinations resulting in 60 experimental units (containers) in each growth chamber. This study was conducted three times in different growth chambers, resulting in 180 total experimental units. A schematic illustration of experimental design and layout of treatments is provided in Fig. C.13.

Two ultradwarf bermudagrass treatments and 10 fungal treatments were used in the study. Two cultivars, Champion and MiniVerde, were included to ascertain cultivar preference characteristics of ERI fungi. Fungal species treatments were as follows: 1 = *G. graminis* var. *graminis* (Ggg); 2 = *G. paulograminis* (Gp); 3 = *M. incrustans* (Mi); 4 = *M. hawaiiensis* (Mh); 5 = *M. cynodontis* (Mc); 6 = *M. taurocanis* (Mt); 7 = *C. cynodontis*

(Cc); 8 = *P. cynodontis* (Pc); 9 = Composite, comprised of equal parts of treatments 1 through 8; and 10 = Untreated Control (UTC). *G. graminis* var. *graminis* and *M. incrustans* were chosen to serve as positive and negative controls, respectively, based on previous research demonstrating their virulence on bermudagrass (Elliott, 1991). UTC treatments were composed of PDA with no fungus.

Statistical analyses were conducted with the GLM procedure in SAS v. 9.3 (SAS Institute Incorporated, Cary, NC, USA). When the F-ratio was significant at the 0.05 level, means were separated using Fisher's protected least significant difference *t* test (SAS Institute Incorporated, Cary, NC, USA). Statistical models for single- and multiple-experiment analyses are provided in Fig 5.2 and Fig. 5.3, respectively.

$$Y_{ijk} = \mu + \alpha_i + \delta_k + \varepsilon_{ik} + \beta_j + (\alpha\beta)_{ij} + e_{ijk};\ \begin{matrix} i=2 \\ j=10 \\ k=3 \end{matrix}$$

Where: $\delta_k \sim N(0,\sigma^2_\delta)$ i.i.d.
$\varepsilon_{ik} \sim N(0,\sigma^2_\varepsilon)$ i.i.d.
$e_{ijk} \sim N(0,\sigma^2)$ i.i.d.
} Independent

Figure 5.2 Statistical model for individual ectotrophic root-infecting (ERI) fungi pathogenicity experiment analyses.

$$Y_{ijkl} = \mu + \delta_k + \gamma_{l(k)} + \alpha_i + \varepsilon_{i(kl)} + \beta_j + (\alpha\beta)_{ij} + e_{ijkl};\ \begin{matrix} i=2 \\ j=10 \\ k=3 \\ l=3 \end{matrix}$$

Where: $\delta_k \sim N(0, \sigma^2_\delta)$
$\gamma_{l(k)} \sim N(0, \sigma^2_\gamma)$
$\varepsilon_{i(kl)} \sim N(0, \sigma^2_\varepsilon)$
$e_{ijkl} \sim N(0, \sigma^2)$
} Independent

Figure 5.3 Statistical model for multiple ectotrophic root-infecting (ERI) fungi pathogenicity experiment analyses.

Data collection

Evaluations were conducted to assess reproducibility of symptoms for summer decline of ultradwarf bermudagrass. Thinning of turf canopy was assessed as total number of nodes (TNN) and gravimetric stolon, rhizome, and foliage weight (GSRFW). Root health and disease were monitored as total number of root-producing nodes (TNRPN), frequency of fungal occurrence (FFO), total root length (TRL), percent disease (PD), and gravimetric root weight (GRW). Upon termination of inoculation experiments (13, 14, and 15 Oct 2014 for experiments 1, 2, and 3, respectively), roots were rinsed with tap water and cleansed of sand particles and debris. Rating methods are described in following sections. Noteworthy, ratings were conducted in the order they are presented.

Total number of nodes

Five plants, equivalent to 25 nodes, were placed in each inoculation container at the beginning of the study. After eight weeks, TNN were assessed by a physical count. These data were considered a summation of the number of nodes from each plant and were recorded as TNN in each container.

Total number of root-producing nodes

The TNRPN was determined in a similar fashion as TNN. Physical assessments were performed to count the number of nodes from which roots were produced. These data were recorded as a composite of all five plants in each container. Samples that did not produce root material were recorded as having zero TNRPN.

Following TNRPN assessments, all root material was severed from stoloniferous and rhizomatous plant material in preparation for subsequent evaluations.

Frequency of fungal occurrence

Roots from each sample were positioned beneath a dissecting microscope and examined for presence of ERI fungal characteristic structures including dark runner hyphae, growth cessation structures, and lobed and simple hyphopodia. If such structures were present, the sample was charted as colonized and given a value of 1; if structures were not present, the sample was recorded as non-colonized and given a value of 0. FFO values for samples that did not produce root material were recorded as missing data due to the inability to assess fungal presence on root material that was not present.

Total root length

Data for TRL were gathered via WinRhizo root scanning software (Regent Instruments Incorporated, Sainte-Foy, Quebec City, Canada). Roots from each sample were placed in waterproof trays (20 × 25 cm) and scanned at 315 dots cm^{-1} (0.032 mm pixel size), in color, using an STD4800 SCANNER. These data were recorded as TRL (cm) for each container. If roots were not produced within a sample, a value of 0 was recorded.

Percent disease

Images acquired for TRL were subjected to PD assessments using a pixel color analysis in WinRhizo. Color classes were established for this study (Fig. C.14). Data generated from WinRhizo that were utilized for PD determination included root surface area occupied by pixels of diseased color classes, referred to as diseased root surface area (DRSA), and root surface area occupied by pixels of healthy color classes, referred to as healthy root surface area (HRSA). Total root surface area (TRSA) was calculated using

the following formula: TRSA = DRSA + HRSA. PD was established via the formula: PD = (DRSA/TRSA) × 100. These data were gathered and recorded for each sample. PD for samples that did not produce root material was recorded as missing data due to the inability to assess disease on root material that was not present.

Gravimetric root weight

Root material from each sample was dried in a Thermo Scientific Precision High-Performance Mechanical Convection Incubator (Precision Scientific Company, Chicago, IL, USA) at 65 C for approximately 72 hours. Dried roots were weighed for gravimetric biomass determination. Data were reported as total GRW for each container. If roots were not produced within a sample, a value of 0 was charted.

Gravimetric stolon, rhizome, and foliage weight

Stoloniferous, rhizomatous, and foliar plant materials were dried in a Thermo Scientific Precision High-Performance Mechanical Convection Incubator at 65 C for approximately 72 hours. Dried samples were weighed for gravimetric biomass determination. Data were recorded as total GSRFW for each container.

Confirmation of Koch's postulates

Koch's postulates were confirmed through a series of isolation and identification steps, similar to those described in Chapter IV. Root material, 5 cm in length, was collected from the uppermost portion of a given root system for each sample, cut into 5 mm sections, surface disinfested in 0.6% sodium hypochlorite solution plus 1 ml 99% ethanol, and rinsed three consecutive times with sterile, distilled water. Surface disinfested roots were allowed to dry, and were plated, at random, onto modified PDA

containing 6 g PDA and 15 g agar liter^{-1} amended with 100 mg chloramphenicol (dissolved in 2.5 ml ethanol) and 100 mg streptomycin sulfate (dissolved in 5.0 ml sterile dH_2O). Plated root tissues were incubated at 25 C until hyphal growth was observed protruding from the surface of the roots. Apical tips of hyphal strands were transferred to PDA (39 g agarose liter^{-1}) at time of emergence. Axenic cultures were incubated at 25 C to allow for maturation of fungal colonies.

Genomic DNA (gDNA) was extracted from vibrant mycelium via manufacturer's instruction for the Fungi/Yeast Genomic DNA Isolation Kit (Norgen Biotek Corporation, Thorold, ON, Canada). Purity and concentration of extracted gDNA was determined by a NanoDrop 2000/2000c (Thermo Fisher Scientific Incorporated, Waltham, MA, USA). The internal transcribed spacer region of the ribosomal RNA genes were subjected to amplification and sequencing. Identification was based on BLAST (Altschul et al., 1990) analyses of resultant sequences.

Results

Analyses of variances for pathogenicity evaluation parameters are provided in Table 5.2. Cultivar by fungal isolate interaction effects were not observed for any parameter. Fungal isolates significantly affected all evaluation parameters in the study. Three of eight evaluation parameters were significantly affected by cultivar selection. Significant differences were observed among experiments for TNRPN, TRL, GRW, and GSRFW; therefore, experiments were analyzed separately for those parameters.

Table 5.2 ANOVA for total number of nodes (TNN), total number of root-producing nodes (TNRPN), frequency of fungal occurrence (FFO), total root length (TRL), percent disease (PD), gravimetric root weight (GRW), and gravimetric stolon, rhizome, and foliage weight (GSRFW) of ultradwarf bermudagrass samples from ectotrophic root-infecting fungi pathogenicity experiments conducted in Starkville, MS.

Source	DF	TNN[†]	TNRPN[‡]	FFO[§]	TRL[¶]	PD[#]	GRW[††]	GSRFW[‡‡]
Experiment	2	NS	⋆⋆	NS	⋆	NS	⋆⋆	⋆
Error 1	4	———	———	———	———	———	———	———
Cultivar (Cv)	1	NS	⋆⋆	NS	⋆	NS	⋆⋆	NS
Error 2	8	———	———	———	———	———	———	———
Fungal species (F)	9	⋆⋆	⋆⋆	⋆⋆⋆	⋆	⋆⋆⋆	⋆	⋆⋆
Cv × F	9	NS	NS	NS	NS	NS	NS	NS
Error 3	144	———	———	———	———	———	———	———

[†] Total number of nodes, assessed by physical count.
[‡] Total number of root-producing nodes, assessed by physical count.
[§] Frequency of fungal occurrence, determined by presence of ERI–like fungal structures.
[¶] Total root length, measured with WinRhizo root scanning software.
[#] Percent disease, assessed with WinRhizo pixel color analysis.
[††] Gravimetric root weight, measured as biomass of dried root material.
[‡‡] Gravimetric stolon, rhizome, and foliage weight, measured as dry biomass.
⋆, ⋆⋆, ⋆⋆⋆ Significant at $p \leq 0.05$, 0.01, and 0.001 levels, respectively.
NS, not significant.

Origin of fungal species

Nineteen turfgrass samples (10.8 cm diameter) were collected from golf courses in five states of the U.S. (Table 5.3). Fungal isolations from colonized roots within the 19 turfgrass samples resulted in 644 axenic cultures, which were assessed for origin. Colony morphology analyses indicated a widespread origin of ERI fungal species. *G. graminis* var. *graminis*, *G. paulograminis*, *M. incrustans*, *M. hawaiiensis*, *M. cynodontis*, *M. taurocanis*, *C. cynodontis*, and *P. cynodontis* were distributed across 2, 4, 2, 5, 3, 3, 1, and 3 states, respectively. Moreover, in numerous instances, multiple fungal species were identified from single turfgrass samples; signifying different fungal populations may be present in concentrated geographical locations.

Table 5.3 Summary of origin and frequency of occurrence for ectotrophic root-infecting (ERI) fungal species and turfgrass samples used in this study, as determined by assessments of colony morphology of axenic fungal cultures.

State	Location	Cultivar	# Isolates[†]	ERI fungal species (number of isolates)
AL	Birmingham	Champion	40	*G. graminis* var. *graminis* (4), *M. cynodontis* (6), *M. hawaiiensis* (1)
HI	Maui	TifEagle	67	*M. cynodontis* (2)
HI	Maui	TifEagle	66	*M. hawaiiensis* (4)
MS	Choctaw	Champion	90	*G. paulograminis* (14), *M. cynodontis* (8), *M. hawaiiensis* (1), *M. incrustans* (12)
MS	Madison	Champion	62	*C. cynodontis* (1), *G. graminis* var. *graminis* (3), *P. cynodontis* (3)
MS	Madison	Miniverde	52	*M. cynodontis* (7)
MS	Starkville	Champion	2	*M. taurocanis* (1)
MS	Starkville	Miniverde	3	*M. incrustans* (2), *M. taurocanis* (1)
MS	Starkville	TifEagle	2	———————————
MS	West Point	Champion	2	*G. paulograminis* (1)
TN	Memphis	Champion	28	*M. hawaiiensis* (2), *P. cynodontis* (4)
TN	Memphis	Champion	9	*G. paulograminis* (1), *M. cynodontis* (2)
TX	Conroe	TifDwarf	56	M. cynodontis (2), *M. hawaiiensis* (1), *M. incrustans* (7), *P. cynodontis* (2)
TX	Houston	TifDwarf	54	*C. cynodontis* (17), *M. cynodontis* (2), *M. hawaiiensis* (1)
TX	Houston	TifEagle	29	*M. cynodontis* (6)
TX	Humble	TifDwarf	33	*G. paulograminis* (2), *M. cynodontis* (9)
TX	Magnolia	TifEagle	13	*M. cynodontis* (1)
TX	Trinity	Champion	28	*C. cynodontis* (2)
TX	Woodlands	Miniverde	8	*M. cynodontis* (3)

[†]Total number of fungal isolates gathered from turfgrass sample, including non-ERI fungal isolates.

Total number of nodes

There were 180 observations for TNN gathered from the three experiments. Experiments were similar ($p = 0.1058$) with respect to mean TNN, and data were combined for analysis (Table 5.2). Fungal species had a significant ($p = 0.0083$) effect on TNN (Tables 5.2 and C.1). All treatments were similar to the UTC except *G. graminis* var. *graminis*, which resulted in a 19% increase in mean TNN compared to the UTC (Fig. 5.4). In contrast to *G. paulograminis*, *C. cynodontis*, *P. cynodontis*, and the composite treatment, *G. graminis* var. *graminis* significantly increased mean TNN by 35, 28, 22, and 23%, respectively (Fig. 5.4). *Magnaporthiopsis* spp. were similar with respect to TNN (Fig. 5.4).

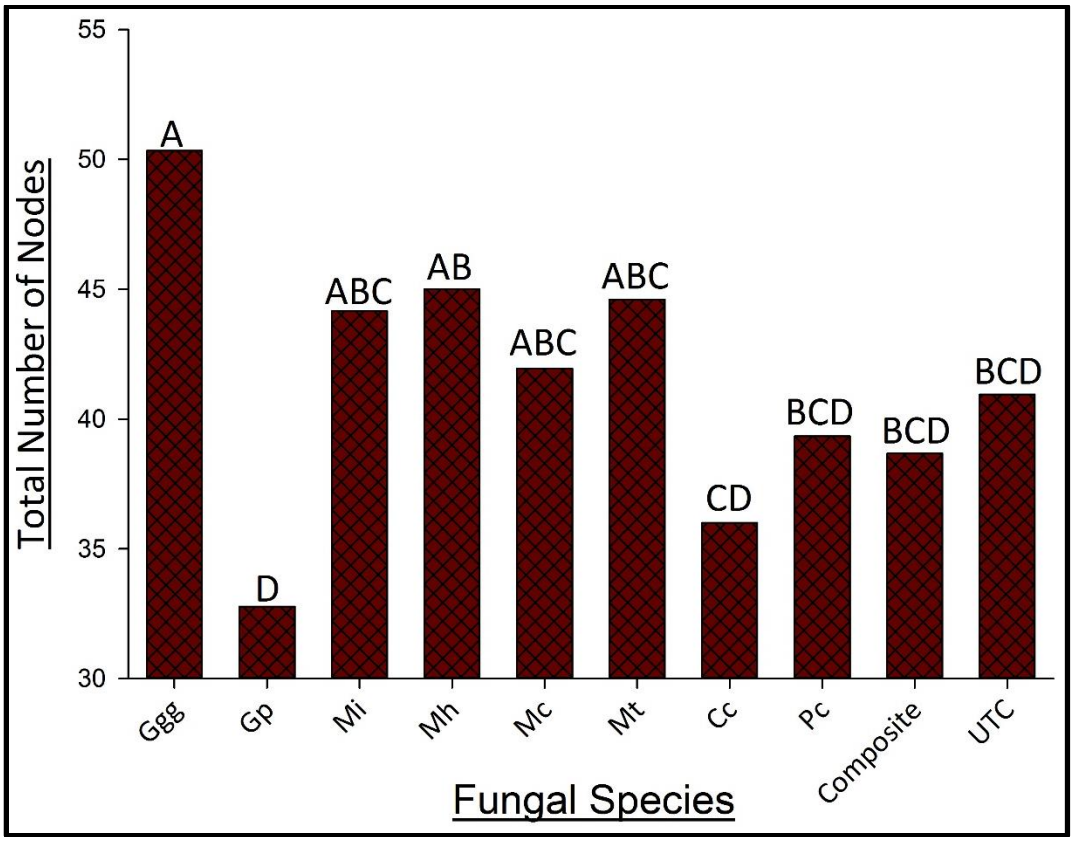

Figure 5.4 Mean total number of nodes (TNN) of Champion and MiniVerde ultradwarf bermudagrass in response to ectotrophic root-infecting fungal species evaluated in pathogenicity experiments 1, 2, and 3 conducted in Starkville, MS.

Bars with the same letter are not significantly different at $p \leq 0.05$, using Fisher's protected LSD.

Total number of root-producing nodes

There were 180 observations for TNRPN gathered from the three experiments. Experiments were significantly ($p = 0.0301$) different with respect to mean TNRPN (Tables 5.2 and C.2). Experiment 1, mean TNRPN = 8.6, had significantly more root-producing nodes, on average, than experiments 2 and 3, which had mean TNRPN = 4.5 and 5.8, respectively. Experiment 1 data was analyzed separately from experiments 2 and 3.

Experiment 1

In experiment 1, mean TNRPN was significantly and independently affected by cultivar ($p = 0.0037$) and fungal species ($p = 0.0386$) (Table 5.4). MiniVerde had 36% more root-producing nodes than Champion (Fig. 5.5). In consideration of fungal species, all treatments were similar to the UTC except *G. paulograminis*, which resulted in a significant 75% reduction of TNRPN (Fig. 5.6). Compared to *M. incrustans*, *M. hawaiiensis*, *M. cynodontis*, *M. taurocanis*, and *P. cynodontis*, *G. paulograminis* significantly reduced mean TNRPN by 83, 78, 76, 82, and 75%, respectively (Fig. 5.6). No differences in TNRPN were observed within the genera *Gaeumannomyces* and *Magnaporthiopsis* (Fig. 5.6).

Table 5.4 ANOVA for total number of root-producing nodes (TNRPN) of Champion and MiniVerde ultradwarf bermudagrass from ectotrophic root-infecting fungi pathogenicity experiment 1 conducted in Starkville, MS.

Source	DF	TNRPN[†]
Cultivar (Cv)	1	⋆⋆
Error 1	2	——
Fungal Species (FS)	9	⋆
Cv × FS	9	NS
Error 3	36	——

[†] Total number of root-producing nodes, assessed by physical count.
⋆,⋆⋆ Significant at $p \leq 0.05$ and 0.01 levels, respectively.
NS, not significant.

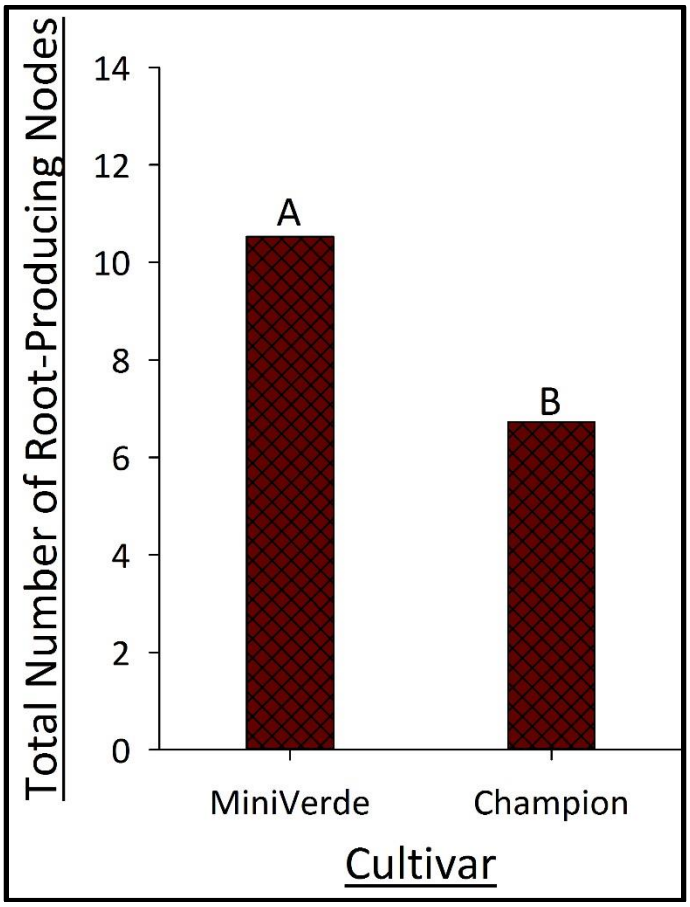

Figure 5.5 Mean total number of root-producing nodes (TNRPN) of ultradwarf bermudagrass in response to cultivar selection from ectotrophic root-infecting fungi pathogenicity experiment 1 conducted in Starkville, MS.

Bars with different letters are significantly different at $p \leq 0.05$, using Fisher's protected LSD.

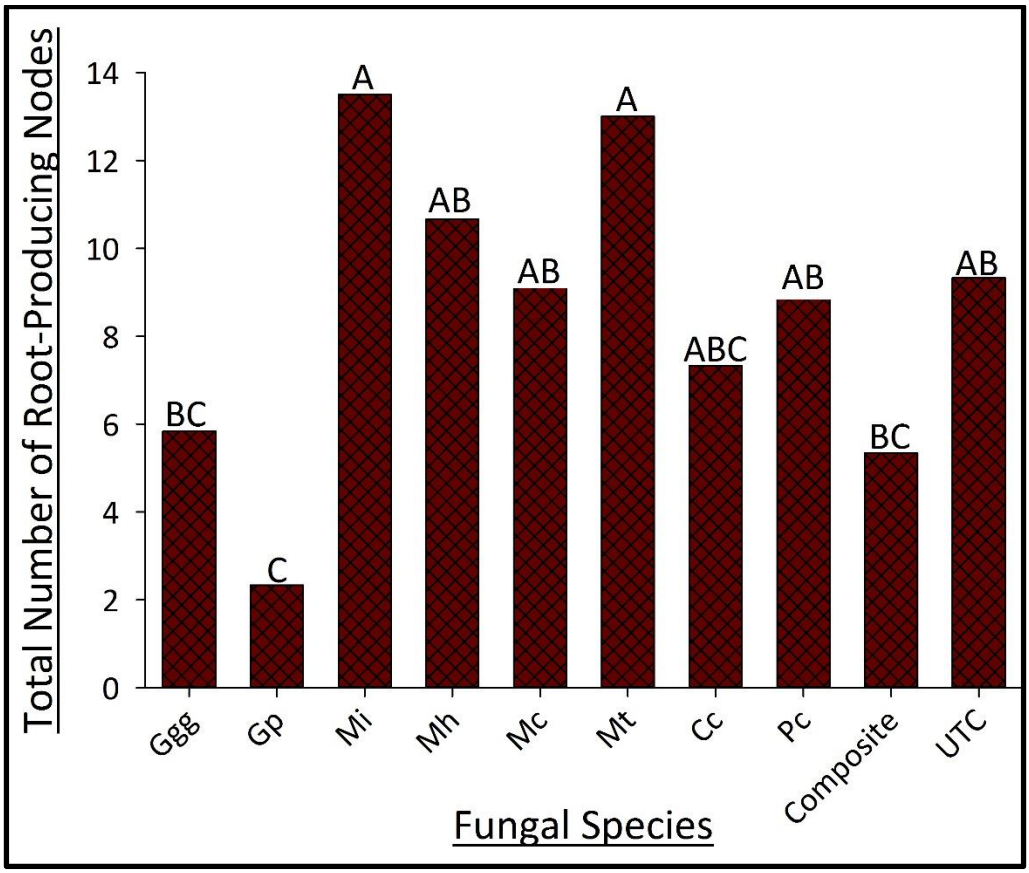

Figure 5.6 Mean total number of root producing nodes (TNRPN) of Champion and MiniVerde ultradwarf bermudagrass in response to ectotrophic root-infecting fungal species evaluated in pathogenicity experiment 1 conducted in Starkville, MS.

Bars with the same letter are not significantly different at $p \leq 0.05$, using Fisher's protected LSD.

Experiments 2 and 3

Experiments 2 and 3 were similar ($p = 0.3588$) with respect to mean TNRPN. Mean TNRPN was significantly and independently affected by cultivar ($p = 0.0492$) and fungal species ($p = 0.0230$) (Table 5.5). MiniVerde had 39% more root-producing nodes than Champion (Fig. 5.8). All treatments were similar to the UTC, with respect to mean TNRPN; however, significant differences among treatments did occur (Fig. 5.8). Compared to *G. graminis* var. *graminis*, *M. cynodontis*, *M. taurocanis*, and *C. cynodontis*, *G. paulograminis* significantly reduced mean TNRPN by 69, 62, 63, and 65%, respectively (Fig. 5.8). *P. cynodontis* significantly reduced mean TNRPN by 54 and 48% compared to *G. graminis* var. *graminis* and *C. cynodontis*, respectively (Fig. 5.8). The composite treatment, compared to *G. graminis* var. *graminis*, *M. taurocanis*, and *C. cynodontis*, resulted in mean root-producing node decreases of 57, 49, and 51%, respectively (Fig. 5.8). *Magnaporthiopsis* spp. were similar with respect to mean TNRPN.

Table 5.5 ANOVA for total number of root-producing nodes (TNRPN) of Champion and MiniVerde ultradwarf bermudagrass from ectotrophic root-infecting fungi pathogenicity experiments 2 and 3 conducted in Starkville, MS.

Source	DF	TNRPN[†]
Cultivar (Cv)	1	⋆
Error 1	2	——
Fungal Species (FS)	9	⋆
Cv × FS	9	NS
Error 3	36	——

[†] Total number of root-producing nodes, assessed by physical count.
⋆ Significant at $p \leq 0.05$ level.
NS, not significant.

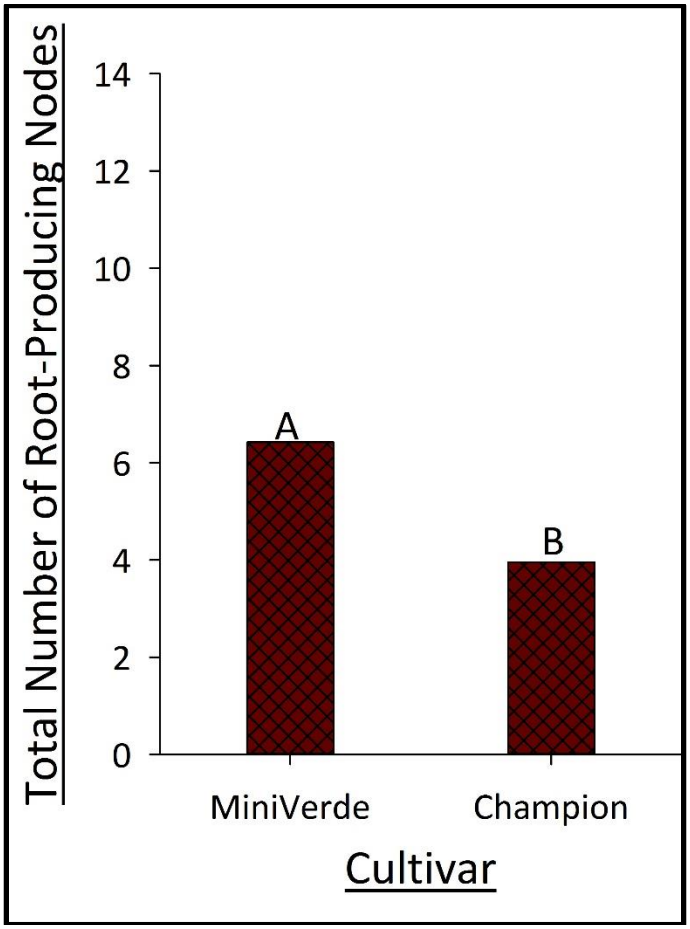

Figure 5.7 Mean total number of root-producing nodes (TNRPN) of ultradwarf bermudagrass in response to cultivar selection from ectotrophic root-infecting fungi pathogenicity experiments 2 and 3 conducted in Starkville, MS.

Bars with different letters are significantly different at $p \leq 0.05$, using Fisher's protected LSD.

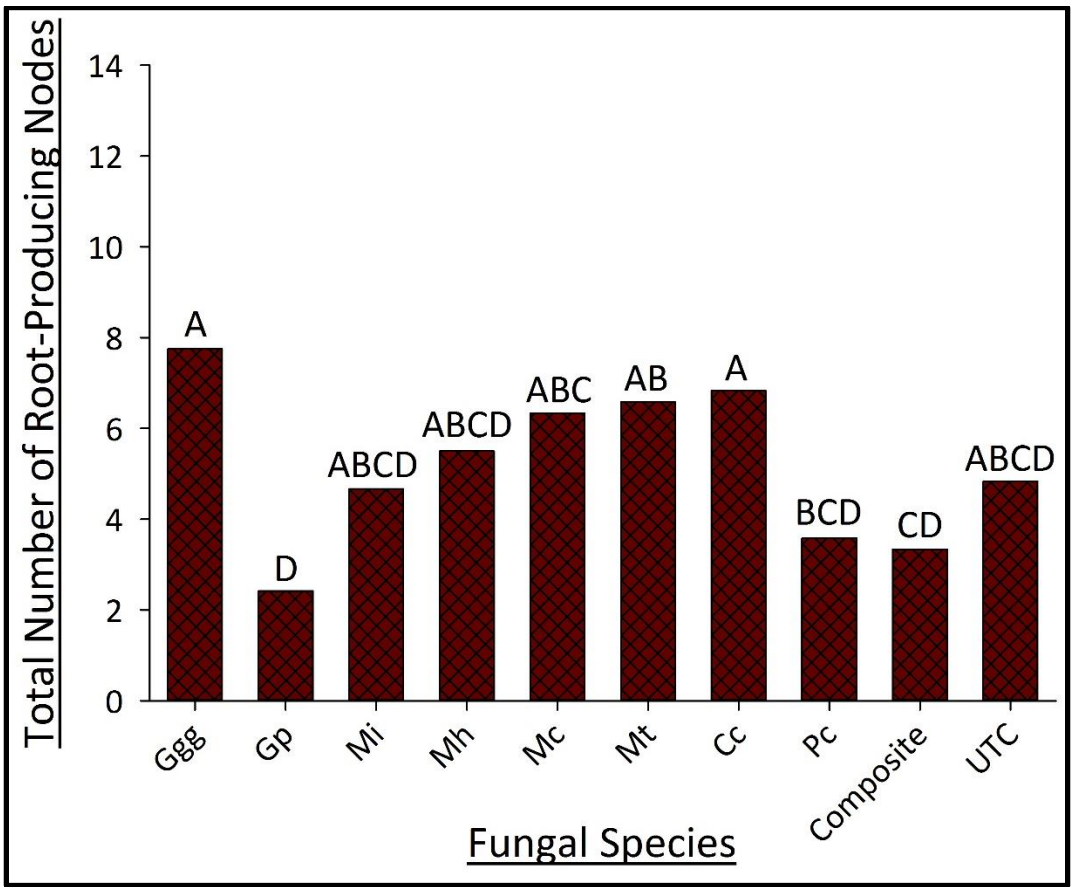

Figure 5.8 Mean total number of root-producing nodes (TNRPN) of Champion and MiniVerde ultradwarf bermudagrass in response to fungal species evaluated in ectotrophic root-infecting fungi pathogenicity experiments 2 and 3 conducted in Starkville, MS.

Bars with the same letter are not significantly different at $p \leq 0.05$, using Fisher's protected LSD.

Frequency of fungal occurrence

Experiments were similar ($p = 0.4444$) with respect to mean FFO; therefore, data were combined for analysis (Table 5.2). FFO ranged from 0 to 100% and was significantly ($p < 0.0001$) affected by fungal species (Tables 5.2 and C.3). All fungal treatments were significantly greater than the UTC (Fig. 5.9). With exception of *P. cynodontis*, which occurred on 6% of samples, each fungal species and the composite occurred on 100% of ultradwarf bermudagrass samples in the three experiments (Fig. 5.9).

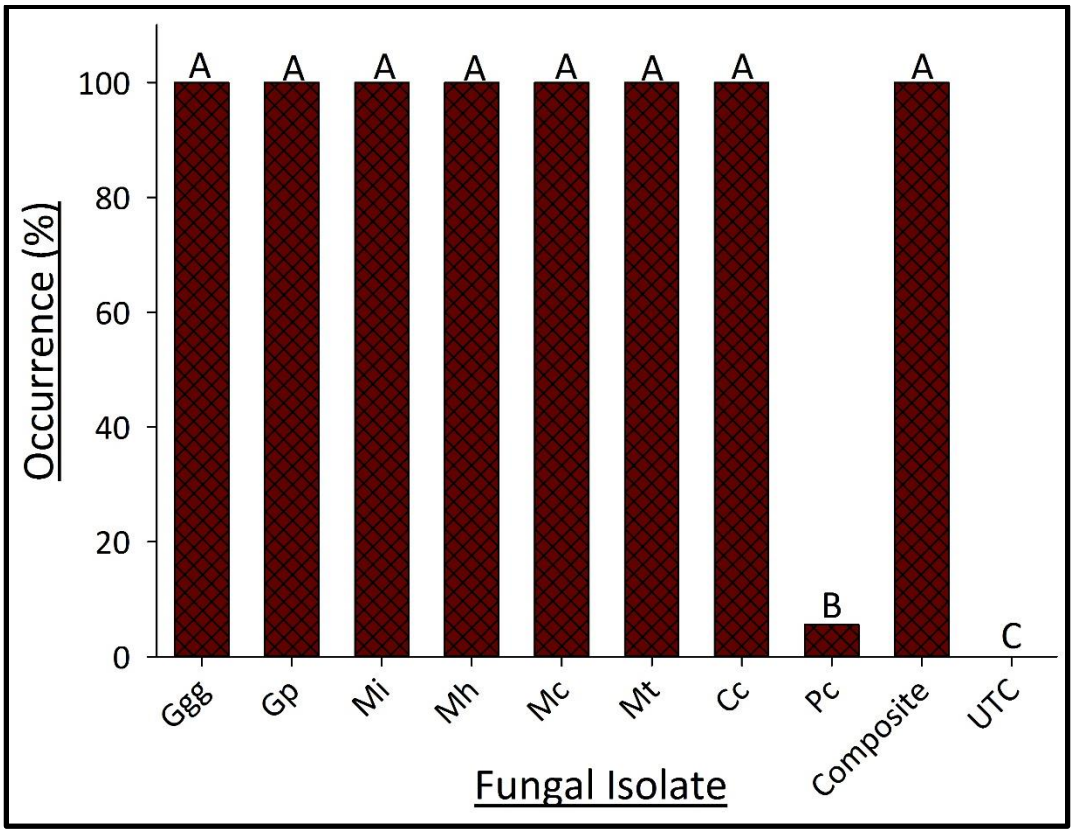

Figure 5.9 Mean frequency of fungal occurrence (FFO) of Champion and MiniVerde ultradwarf bermudagrass in response to ectotrophic root-infecting fungal species evaluated in pathogenicity experiments 1, 2, and 3 conducted in Starkville, MS.

Bars with the same letter are not significantly different at $p \leq 0.05$, using Fisher's protected LSD.

Total root length

There were 180 observations for TRL gathered from the three experiments. Experiments were significantly ($p = 0.0412$) different with respect to mean TRL (Tables 5.2 and C.4). Experiment 1, mean TRL = 1,754 cm, had significantly more TRL, on average, than experiments 2 and 3, which had mean TRL = 939 and 1,034 cm, respectively. Experiment 1 data were analyzed separately from experiments 2 and 3.

Experiment 1

In experiment 1, mean TRL was significantly ($p = 0.0337$) affected by fungal species (Table 5.6). All fungal species were similar to the UTC except *M. hawaiiensis*, which had significantly greater mean TRL (Fig. 5.10). *G. paulograminis* had the least TRL, numerically, and resulted in significant mean TRL reductions of 86, 87, 80, 80, and 83% compared to *M. incrustans*, *M. hawaiiensis*, *M. cynodontis*, *M. taurocanis*, and *P. cynodontis*, respectively (Fig. 5.10). Mean TRL of *G. graminis* var. *graminis* treatments were significantly reduced by 46% compared to *M. hawaiiensis* (Fig. 5.10). In contrast to *M. hawaiiensis*, *C. cynodontis* resulted in a significant mean TRL reduction of 52% (Fig. 5.10). No differences were observed within the genera *Gaeumannomyces* and *Magnaporthiopsis* (Fig. 5.10).

Table 5.6 ANOVA for total root length (TRL) of Champion and MiniVerde ultradwarf bermudagrass from ectotrophic root-infecting fungi pathogenicity experiment 1 conducted in Starkville, MS.

Source	DF	TNRPN[†]
Cultivar (Cv)	1	NS
Error 1	2	——
Fungal Species (FS)	9	⋆
Cv × FS	9	NS
Error 3	36	——

[†] Total number of root-producing nodes, assessed by physical count.
⋆ Significant at $p \leq 0.05$ level.
NS, not significant.

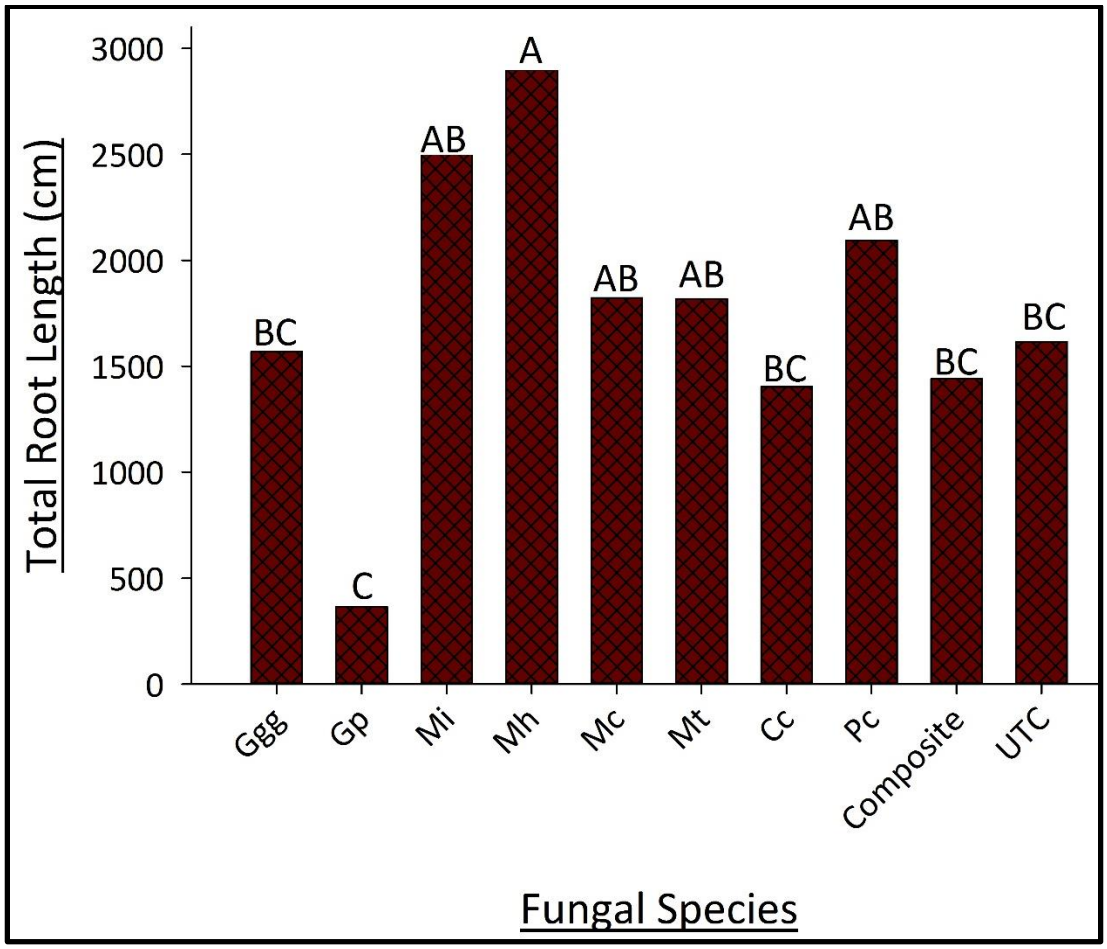

Figure 5.10 Mean total root length (TRL) of Champion and MiniVerde ultradwarf bermudagrass in response to ectotrophic root-infecting fungal species evaluated in pathogenicity experiment 1 conducted in Starkville, MS.

Bars with the same letter are not significantly different at $p \leq 0.05$, using Fisher's protected LSD.

Experiments 2 and 3

Experiments 2 and 3 were similar ($p = 0.7709$) with respect to mean TRL and data were combined for analysis. There were no differences observed among treatments with respect to mean TRL of combined data from experiments 2 and 3.

Percent disease

Experiments were similar ($p = 0.6730$) with respect to mean PD; therefore, data were combined for analysis (Table 5.2). PD ranged from 0 to 27% and was significantly ($p < 0.0001$) affected by fungal species (Tables 5.2 and C.5). All fungal treatments resulted in significantly greater mean PD than the UTC treatment. Excluding the composite, which is made of equal portions of all eight fungal species, fungal treatments resulted in two levels of virulence. *G. graminis var. graminis*, *M. incrustans*, *M. hawaiiensis*, *M. cynodontis*, *M. taurocanis*, and *P. cynodontis* resulted in reduced disease severity compared to *G. paulograminis* and *C. cynodontis* (Fig. 5.11). The composite treatment was similar to each of the separate fungal species, with respect to mean PD (Fig. 5.11).

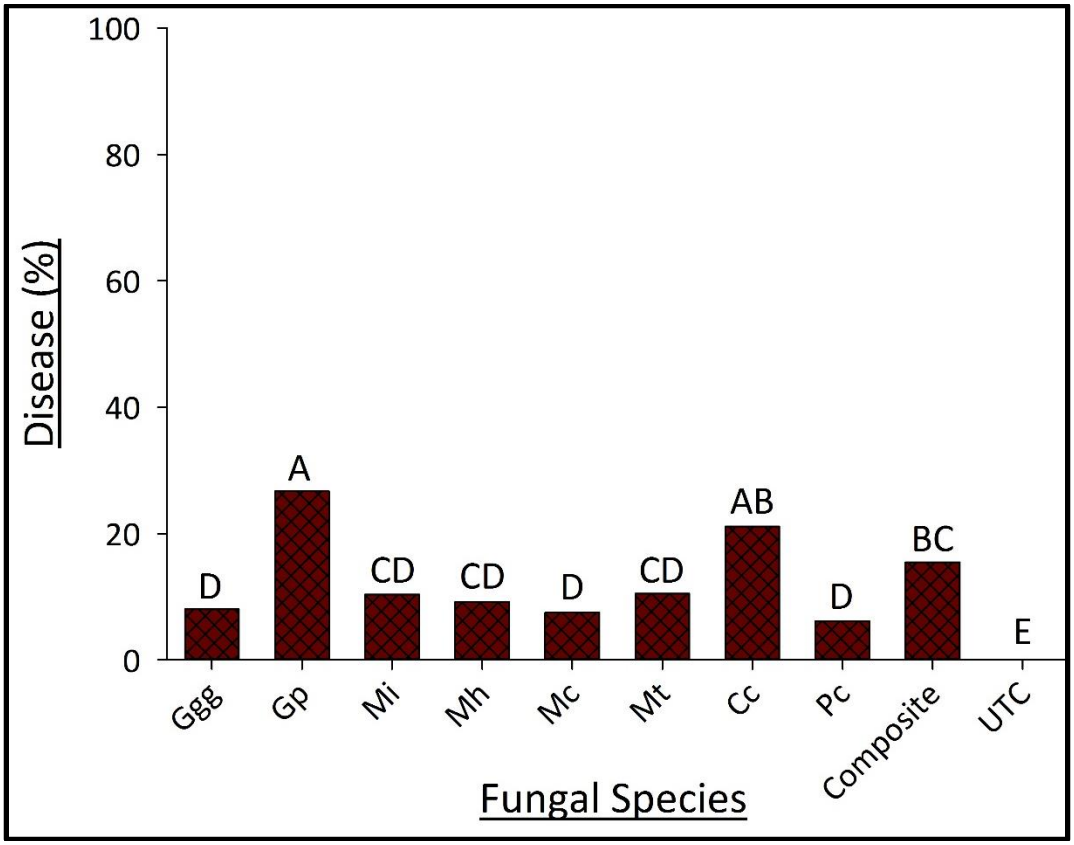

Figure 5.11 Mean percent disease (PD) of Champion and MiniVerde ultradwarf bermudagrass in response to ectotrophic root-infecting fungal species evaluated in pathogenicity experiments 1, 2, and 3 conducted in Starkville, MS.

Bars with the same letter are not significantly different at $p \leq 0.05$, using Fisher's protected LSD.

Gravimetric root weight

There were 180 observations for GRW gathered from the three experiments. Experiments were significantly ($p = 0.0074$) different with respect to mean GRW (Tables 5.2 and C.6). Experiment 1, mean GRW = 52.5 mg, had significantly greater root mass, on average, than experiments 2 and 3, which had mean GRW = 26.3 and 21.8 mg, respectively. Experiment 1 data were analyzed separately from experiments 2 and 3.

Experiment 1

In experiment 1, mean GRW was significantly ($p = 0.0095$) affected by fungal species (Table 5.7). All treatments were similar to the UTC, with respect to mean GRW (Fig. 5.12). *G. paulograminis* had the least GRW, numerically, and resulted in significant mean GRW reductions of 92, 88, 91, and 89% compared to *M. incrustans*, *M. hawaiiensis*, *M. taurocanis*, and *P. cynodontis*, respectively (Fig. 5.12). Mean GRW of *G. graminis* var. *graminis* treatments were significantly reduced by 71 and 69% compared to *M. incrustans* and *M. taurocanis*, respectively (Fig. 5.12). In contrast to *M. incrustans* and *M. taurocanis*, *C. cynodontis* resulted in significant mean GRW reductions of 56 and 53%, respectively (Fig. 5.12).

Table 5.7 ANOVA for gravimetric root weight (GRW) of Champion and MiniVerde ultradwarf bermudagrass from ectotrophic root-infecting fungi pathogenicity experiment 1 conducted in Starkville, MS.

Source	DF	TNRPN[†]
Cultivar (Cv)	1	NS
Error 1	2	——
Fungal Species (FS)	9	⋆⋆
Cv × FS	9	NS
Error 3	36	——

[†] Total number of root-producing nodes, assessed by physical count.
⋆⋆ Significant at $p \leq 0.01$ level.
NS, not significant.

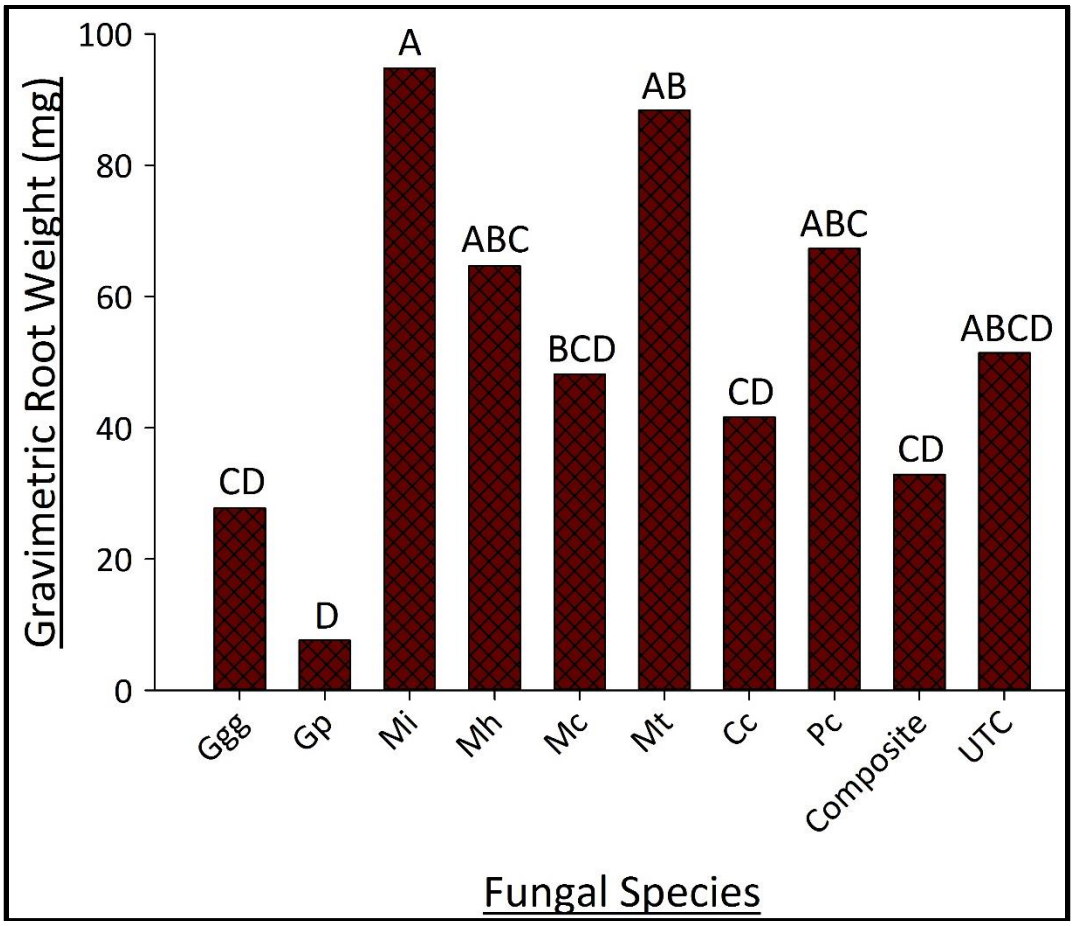

Figure 5.12 Mean gravimetric root weight (GRW) of Champion and MiniVerde ultradwarf bermudagrass in response to ectotrophic root-infecting fungal species evaluated in pathogenicity experiment 1 conducted in Starkville, MS.

Bars with the same letter are not significantly different at $p \leq 0.05$, using Fisher's protected LSD.

Experiments 2 and 3

Experiments 2 and 3 were similar ($p = 0.5890$) with respect to mean GRW; therefore data were combined for analysis. Cultivars significantly ($p = 0.0161$) affected mean GRW (Table 5.8). MiniVerde, mean GRW = 35.9 mg, had significantly more root mass than Champion, which had mean GRW = 12.3 mg.

Table 5.8 ANOVA for gravimetric root weight (GRW) of Champion and MiniVerde ultradwarf bermudagrass from ectotrophic root-infecting fungi pathogenicity experiments 2 and 3 conducted in Starkville, MS.

Source	DF	TNRPN[†]
Cultivar (Cv)	1	⋆
Error 1	2	——
Fungal Species (FS)	9	NS
Cv × FS	9	NS
Error 3	36	——

[†] Total number of root-producing nodes, assessed by physical count.
⋆ Significant at $p \leq 0.05$ level.
NS, not significant.

Gravimetric stolon, rhizome, and foliage weight

There were 180 observations for GSRFW gathered from the three experiments. Experiments were significantly ($p = 0.0185$) different with respect to mean GSRFW (Tables 5.2 and C.7). Experiment 1, mean GSRFW = 248.1 mg, had significantly greater stolon, rhizome, and foliage mass, on average, than experiments 2 and 3, which had mean GSRFW = 156.3 and 174.1 mg, respectively. Experiment 1 data were analyzed separately from experiments 2 and 3.

Experiment 1

There were no differences observed among treatments in experiment 1 with respect to mean GSRFW.

Experiments 2 and 3

Experiments 2 and 3 were similar ($p = 0.4595$) with respect to mean GSRFW and data were combined for analysis. Mean GSRFW was significantly ($p = 0.0055$) affected by fungal species (Table 5.9). All treatments were similar except for *G. graminis* var. *graminis*, which had significantly greater GSRFW, on average, than other treatments (Fig. 5.13). *G. graminis* var. *graminis* resulted in significant mean GSRFW increases of 52, 42, 26, 28, 27, 42, 43, 52 and 52% compared to *G. paulographaminis*, *M. incrustans*, *M. hawaiiensis*, *M. cynodontis*, *M. taurocanis*, *C. cynodontis*, *P. cynodontis*, composite, and UTC treatments, respectively (Fig. 5.13).

Table 5.9 ANOVA for gravimetric stolon, rhizome, and foliage weight (GSRFW) of Champion and MiniVerde ultradwarf bermudagrass from ectotrophic root-infecting fungi pathogenicity experiments 2 and 3 conducted in Starkville, MS.

Source	DF	TNRPN[†]
Cultivar (Cv)	1	NS
Error 1	2	——
Fungal Species (FS)	9	⋆⋆
Cv × FS	9	NS
Error 3	36	——

[†] Total number of root-producing nodes, assessed by physical count.
⋆⋆ Significant at $p \leq 0.01$ level.
NS, not significant.

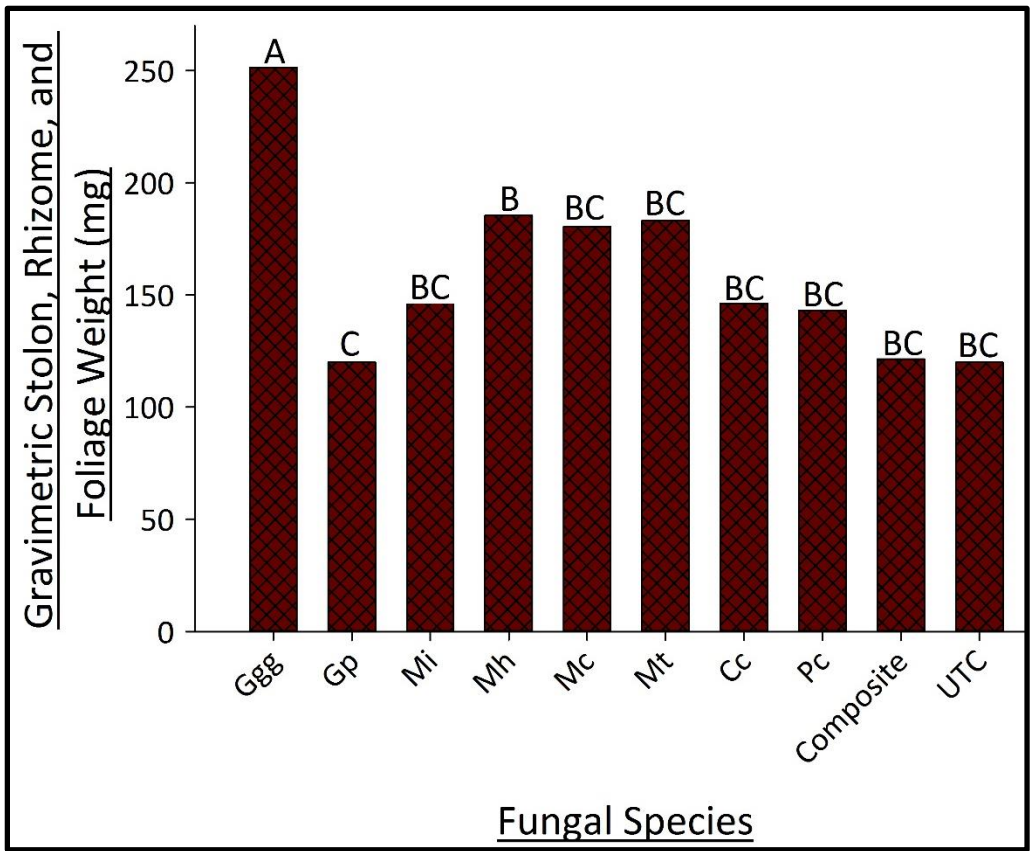

Figure 5.13 Mean gravimetric stolon, rhizome, and foliage weight (GSRFW) of Champion and MiniVerde ultradwarf bermudagrass in response to ectotrophic root-infecting fungal species evaluated in pathogenicity experiments 2 and 3 conducted in Starkville, MS.

Bars with the same letter are not significantly different at $p \leq 0.05$, using Fisher's protected LSD.

Discussion

This study provides insight to the theory that novel ERI fungi elicit the occurrence of summer decline on ultradwarf bermudagrass putting greens. Summer decline is characterized by a thinning and loss of foliar plant material, which can progress into substantial turf loss, resulting in large areas of exposed soil. Roots of affected plants appear diminutive in size, brown to black in color, and are commonly colonized with dark, runner hyphae, which is characteristic of ERI fungi. Assessments pertaining to foliar characteristics included TNN and GSRFW; effects on root health and disease were monitored as TNRPN, FFO, TRL, PD, and GRW.

Regarding evaluation parameters, differences were observed between cultivars, fungal species, and experiments. Three experiments were conducted and inadvertent variations in lighting conditions existed among them. Light exposure in experiment 1 was 12 and 11% greater than experiments 2 and 3, respectively, and resulted in increased biomass production. Previous studies demonstrated that root material and foliar density of bermudagrass decrease when subjected to reduced light environments (Miller, et al., 2005; Baldwin and McCarty, 2008). This provides plausible justification for differences observed among experiments with respect to TNRPN, TRL, GRW, and GSRFW evaluation parameters. Henceforth, experiment 1 is referenced as 'higher light environment' and experiments 2 and 3 are referred to as 'lower light environment'.

Cultivars differed with respect to TNRPN and GRW; however, fungal species behaved similarly on both cultivars, across all evaluation parameters in this study. This suggests ERI fungi do not exhibit host preference between Champion and MiniVerde cultivars. In consideration of GRW, no difference was observed between cultivars

exposed to a higher light environment, but MiniVerde was superior in lower light environments. Furthermore, MiniVerde had greater mean TNRPN than Champion regardless of lighting conditions. This information is trivial in regards to pathogenicity of ERI fungi, but suggests MiniVerde may have enhanced morphological characteristics, compared to Champion.

Although Koch's postulates were completed for each of the six novel ERI fungal species, as well as the two recognized ERI fungal species, varying levels of virulence were observed. Likewise, a prior assessment revealed a diversity in virulence among four ERI fungi, namely, *G. cylindrosporus, M. incrustans, M. poae*, and *Phialophora graminicola* (anamorph of *G. cylindrosporus*), when inoculated into Kentucky bluegrass and annual bluegrass turf (Landschoot and Jackson, 1990).

C. cynodontis and *G. paulograminis* were the most destructive and aggressive ERI fungi evaluated in this study. *G. paulograminis* produced characteristic rounded, moderately lobed hyphopodia, and was readily observed on roots as well as stoloniferous material. Plants inoculated with *G. paulograminis* consistently had the least TNN, TNRPN, TRL, GRW, and GSRFW. Additionally, *G. paulograminis* incited the greatest percent disease of all ERI fungi in the study. Visually, *C. cynodontis* appeared slightly less destructive than *G. paulograminis*; however, evaluation parameters suggest the two fungal species behaved in a similar manner. Like *G. paulograminis*, *C. cynodontis* readily produced characteristic hyphopodia, which were rounded to mitten-shaped, and was frequently observed on roots as well as stolons.

Findings from this evaluation were somewhat dissimilar to an earlier study by Elliott (1991). *G. graminis* var. *graminis* was moderately to weakly virulent to

bermudagrass in this assessment, whereas Elliott (1991) reported *G. graminis* var. *graminis* to be highly destructive to bermudagrass. Furthermore, *G. graminis* var. *graminis*-treated plants displayed enhanced foliar properties in current studies, which suggest this fungal species is not the primary causal agent of foliar symptomatology associated with summer decline of ultradwarf bermudagrass putting greens. *G. graminis* var. *graminis* was consistently isolated from root material, and characteristic deeply lobed hyphopodia were produced on stolons and leaf sheaths of inoculated plants.

Also contrasting to prior evaluations, moderate to weak virulence was observed among *M. incrustans*-treated plants in this study, while *M. incrustans* did not affect bermudagrass in the earlier study by Elliott (1991). *M. incrustans* was similar to other species of *Magnaporthiopsis*, and all were readily isolated from inoculated plants. Unlike *C. cynodontis*, *G. graminis* var. *graminis*, and *G. paulograminis*, species of *Magnaporthiopsis* did not produce hyphopodia and were not observed on ultradwarf bermudagrass stolons. Prior to recent studies at MSU, summarized in Chapter IV, a *Magnaporthiopsis*, excluding *M. incrustans*, had not been isolated from bermudagrass in nature; however, pathogenicity of a creeping bentgrass (*Agrostis stolonifera* L.) derived isolate of *M. poae* to bermudagrass was demonstrated in a preceding evaluation (Elliott, 1991). Nonetheless, subsequent information from this study reveals a moderate to weak level of virulence among species of *Magnaporthiopsis*, concerning ultradwarf bermudagrass.

P. cynodontis, like species of *Magnaporthiopsis*, did not produce hyphopodia, and was not observed on stoloniferous material. *P. cynodontis* was the least-aggressive ERI fungal species in the evaluation with respect to occurrence on roots of inoculated

ultradwarf bermudagrass plants. The genus *Pseudophialophora* is typified by *P. eragrostis*, which was identified from healthy roots of Poaceae plants (Luo et al., 2014). As suggested in Chapter IV, relatedness to non–pathogenic *Pseudophialophora* spp. provides a reasonable theory for decreased activity and presence of *P. cynodontis* on roots of ultradwarf bermudagrass.

Overall, ERI fungi were not entirely devastating to ultradwarf bermudagrass plants, and a broad view of summer decline must be considered to understand this reasoning. Summer decline is most prevalent in the Deep South throughout late summer and early fall months. During this period, daytime temperatures often exceed 38 C, which surpasses the upper limit for optimal growth of bermudagrass (McCarty and Miller, 2002). Extreme temperatures, coupled with increased foot and vehicular traffic, decreased mowing heights, and biological stress factors such as nematodes, insects, and other fungi can often debilitate ultradwarf bermudagrasses at this time of year (Johnson, 1970; Giblin-Davis et al., 1992; Bunnell et al., 2005; Trappe et al., 2011). Growth chamber environments in these pathogenicity evaluations were not stressful to bermudagrass plants; instead, conditions were near optimal for turf growth. Mean daytime air temperatures were less than 34 C, plants did not experience mechanical or foot traffic stress, and additional biological stresses were absent.

This study supports the concept that summer decline is caused by a complex of stress factors and not by ERI fungi alone. *In vivo* evaluations of ERI fungi were successful in recreating symptoms associated with summer decline, especially within root systems; however, not as severe as observed by golf course superintendents and plant disease diagnosticians. This is likely because plants in this study were grown at near

optimal conditions; whereas, in nature, ultradwarf bermudagrass plants exhibiting symptoms of summer decline are weakened by many stress factors, and increasingly vulnerable to ERI fungi.

Another important aspect to this study was general geographic origins for ERI fungi. Many fungal species were isolated from a number of turfgrass samples, submitted from various locations across the southern U.S. and Hawaii. This implies a possible widespread distribution of these fungi across the southern U.S. Furthermore, multiple ERI fungi were isolated from roots within a single turfgrass sample. This suggests a cohabitant nature among ERI fungi, a theory that should be studied further.

In conclusion, findings from this research necessitate subsequent investigations concerning effects of ERI fungi on ultradwarf bermudagrass turf managed to golf course standards as well as distribution of ERI fungi across the southern U.S. To gain a more precise knowledge of the role ERI fungi play in summer decline, real-world conditions must be considered. A proposed evaluation scheme includes soil inoculations of ERI fungi into established ultradwarf bermudagrass turf. Plots should be managed to golf course standards, which would include daily mowings at 3 to 4 mm height of cut, irrigation to prevent foliar wilt, and fertilization according to soil tests. Additionally, foot traffic should be applied to supply surface stress and root zone compaction. Suggestions for distribution analyses include a strategic sampling of ultradwarf bermudagrass root systems for presence of ERI fungal structures, isolation of fungal organisms, and molecular identification of subsequent axenic cultures.

Future studies may include establishing methods for control, determining turfgrass host range, identifying modes of dissemination, and developing rapid diagnostic tools for ERI fungal species.

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APPENDIX A

SUPPLEMENTARY MATERIAL FOR CULTURAL MANAGEMENT STUDIES

Figure A.1 Overview of ultradwarf bermudagrass research plots at the R. R. Foil Plant Science Research Center.

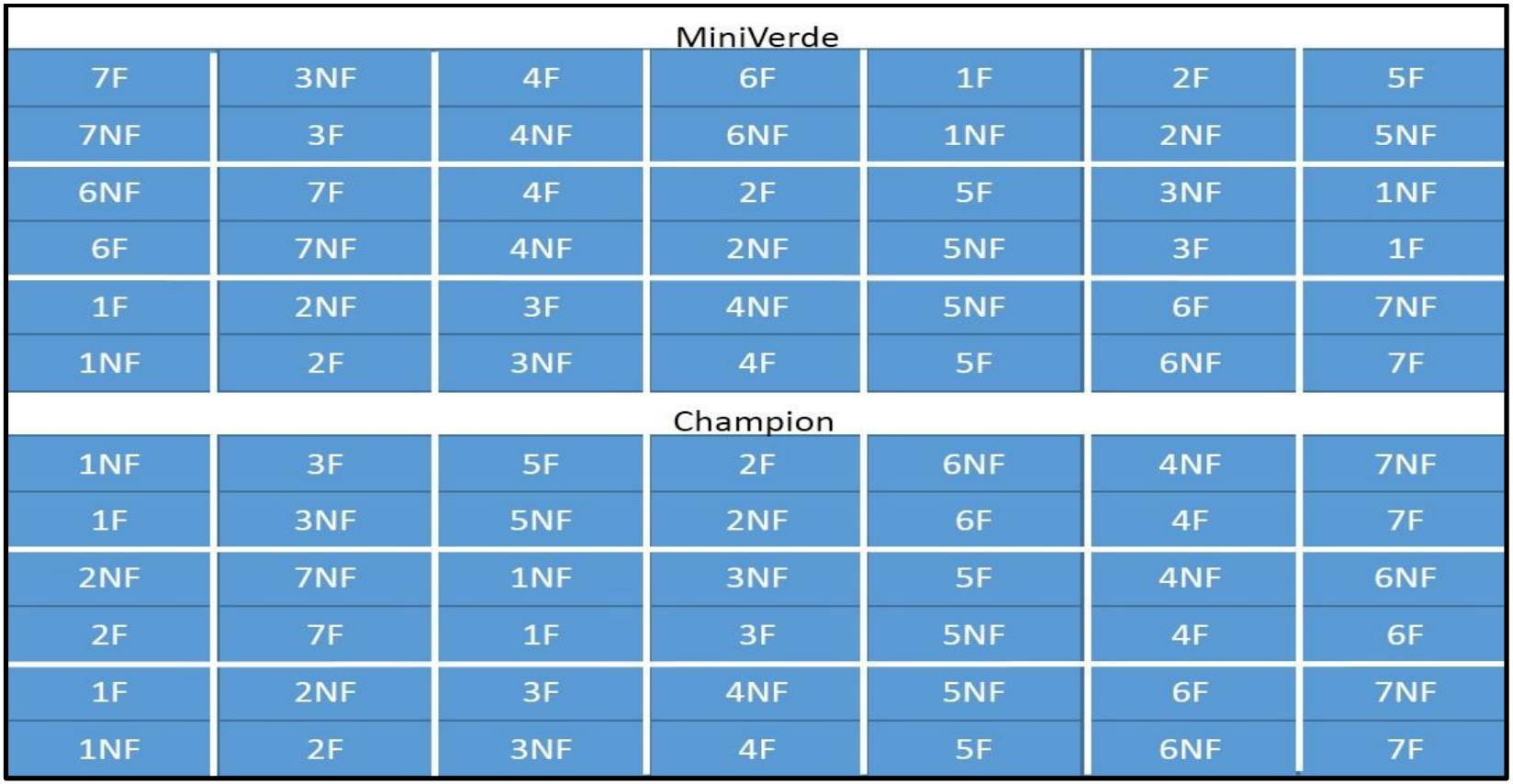


Figure A.2 Schematic illustration of experimental design and layout of cultural practice treatments and seasonal fungicide program.

Main-plots are presented as blue boxes separated into sub-plots by black horizontal lines. 1 = VM, 2 = VM+SL, 3 = VM+SP, 4 = VM+SC, 5 = SL, 6 = SP, 7 = SC, F = presence of seasonal fungicide program, NF = absence of seasonal fungicide program.

Table A.1 Application timing of weekly cultural practices. Treatments were applied at corresponding weeks in June, July, August, and September of 2012 and 2013. Initial treatments were applied 8 Jun 2012 and 12 Jun 2013. Concluding treatments were applied 14 Sep 2012 and 18 Sep 2013.

	Cultural Practice Treatment						
Week	**VM**	**VM + SL**	**VM + SP**	**VM + SC**	**SL**	**SP**	**SC**
1	VM	VM	VM	VM	—	—	—
2	VM	VM	VM	VM	—	—	—
3	VM	SL	SP	SC	SL	SP	SC
4	VM	VM	VM	VM	—	—	—

Figure A.3 Initiation of treatment applications for cultural management study, 8 June 2012.

Figure A.4 Vacu-Cutter™ insert (True-Surface® Greens Care Collection).

Figure A.5 Deep Slicer insert (True-Surface® Greens Care Collection).

Figure A.6 Greens Spiker insert (True-Surface® Greens Care Collection).

Figure A.7 Vacu-Scarifier™ insert (True-Surface® Greens Care Collection).

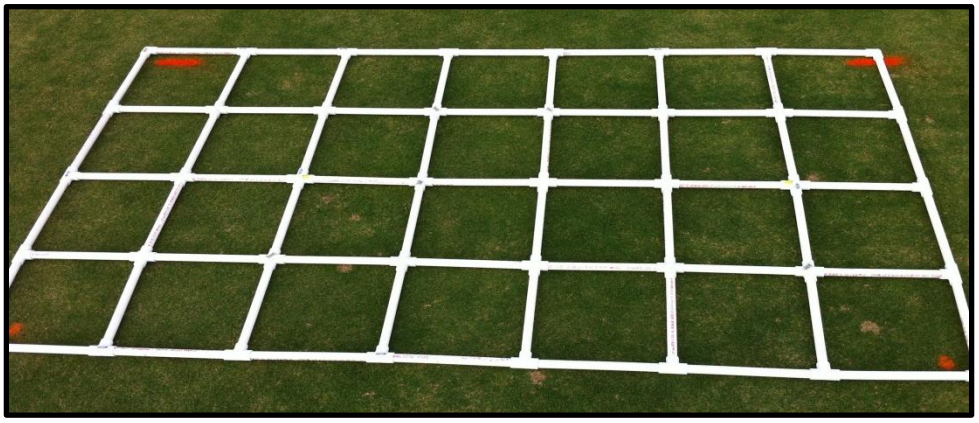


Figure A.8 Grid placement for cultural management study data collection.

APPENDIX B

SUPPLEMENTARY MATERIAL FOR IDENTIFICATION AND CHARACTERIZATION STUDIES

Figure B.1 Field symptomatology of summer decline on an ultradwarf bermudagrass putting green.

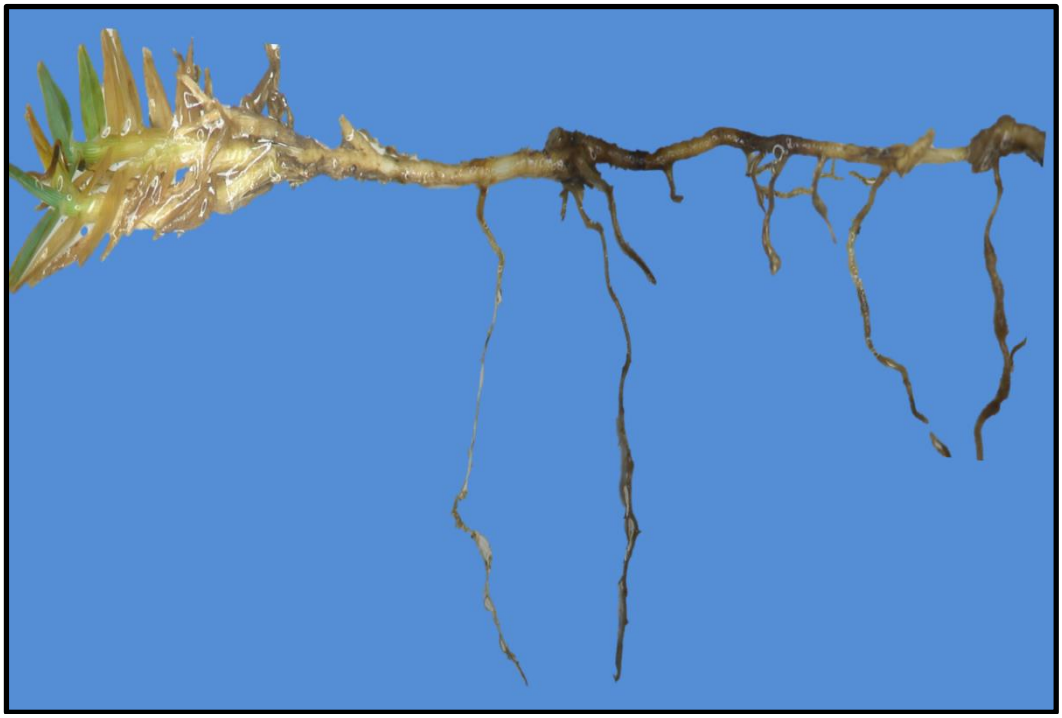


Figure B.2 Symptomatology of an ultradwarf bermudagrass plant affected by summer decline.

Figure B.3 Ultradwarf bermudagrass root material colonized by dark, runner hyphae.

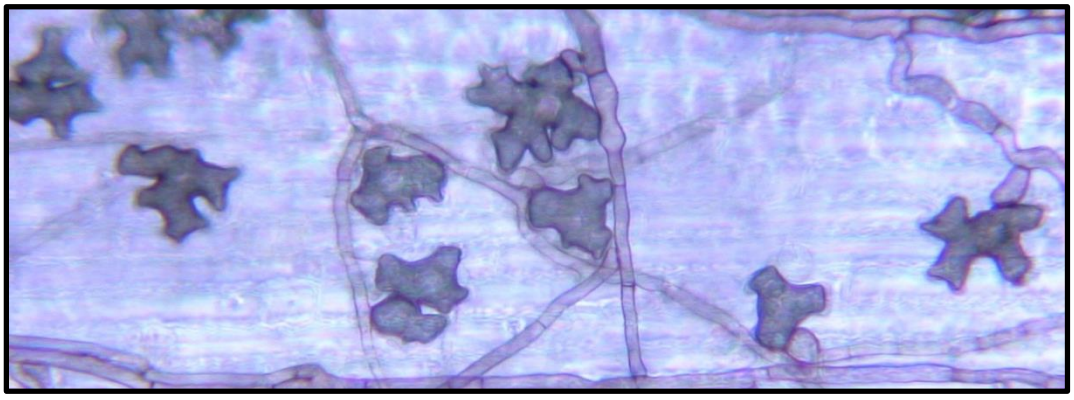


Figure B.4 Lobed hyphopodia of an ectotrophic root-infecting fungus.

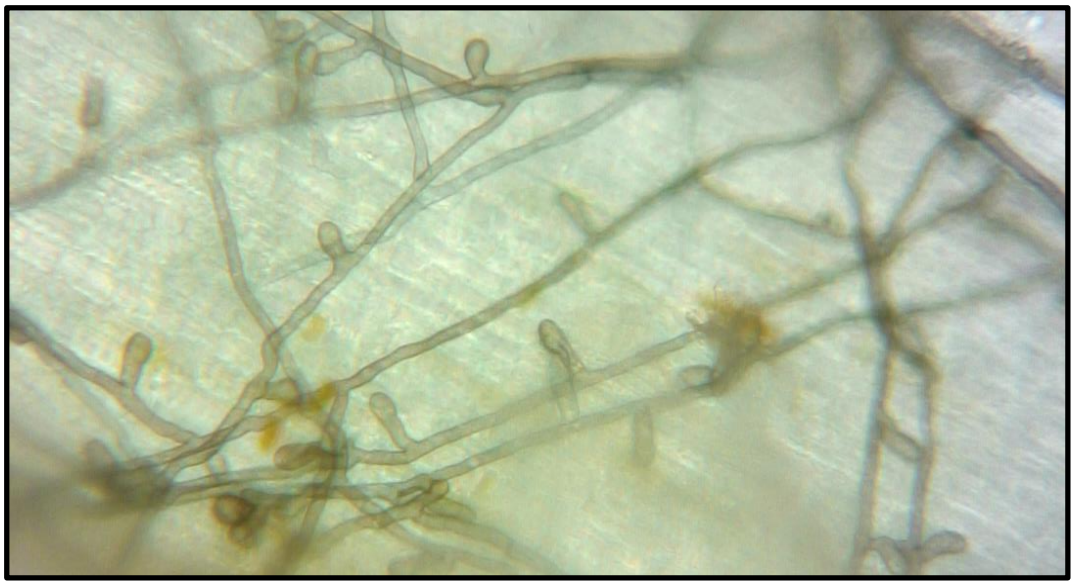


Figure B.5 Simple hyphopodia of an ectotrophic root-infecting fungus.

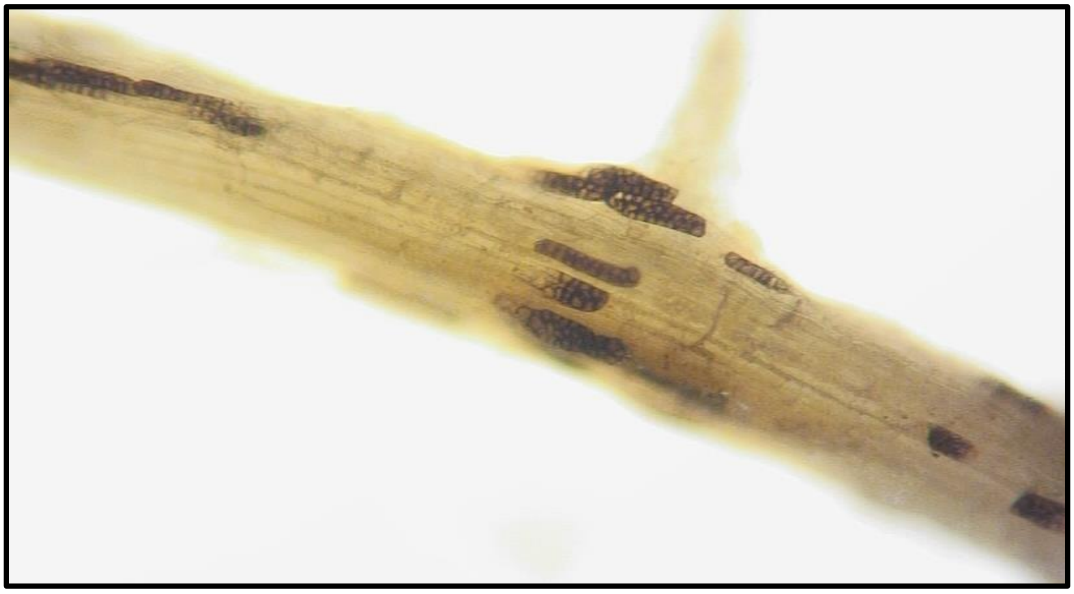


Figure B.6 Growth cessation structures of an ectotrophic root-infecting fungus.

Table B.1 Summary of Basic Local Alignment Search Tool analyses for internal transcribed spacer region of ectotrophic root-infecting fungal isolates used in molecular and phylogenetic studies.

Isolate ID	Query Accession	Best match in BLAST analysis	Subject Accession	Identity score (% similarity)
AD1–2	KJ855487	*Gaeumannomyces graminis* var. *graminis*	AY428781	529/537 (99)
DR1–4	KJ855491	*Gaeumannomyces graminis* var. *graminis*	AY428780	536/539 (99)
DR6–4	KJ855492	*Gaeumannomyces graminis* var. *graminis*	AY428780	536/539 (99)
DR10–3	KJ855488	*Gaeumannomyces graminis* var. *graminis*	AY428780	536/539 (99)
DR12–1	KJ855489	*Gaeumannomyces graminis* var. *graminis*	AY428780	537/539 (99)
DR13–1	KJ855490	*Gaeumannomyces graminis* var. *graminis*	AY428780	537/539 (99)
GSGC10–2	KJ855493	*Magnaporthiopsis poae*	AJ010042	362/383 (95)
GSGC15–3	KJ855494	*Gaeumannomyces graminis* var. *graminis*	AY428781	529/537 (99)
GSGC15–4	KJ855495	*Gaeumannomyces graminis* var. *graminis*	AY428781	529/537 (99)
HCC3–4	KJ855496	*Magnaporthiopsis poae*	AJ010042	361/383 (94)
HP24–3	KJ855497	*Gaeumannomyces cylindrosporus*	U17211	402/427 (94)
HP38–4	KJ855498	*Gaeumannomyces cylindrosporus*	U17211	402/427 (94)
KR10–6	KJ855499	*Magnaporthiopsis poae*	AF074406	362/384 (94)
LC8–6	KJ855500	*Magnaporthiopsis incrustans*	JF414846	529/540 (98)
OW4–4	KJ855501	*Gaeumannomyces graminis* var. *graminis*	AY428780	537/539 (99)
RRFCHMP1–3	KJ855502	*Magnaporthiopsis incrustans*	JF414846	499/537 (93)
RRFMV10–2	KJ855503	*Magnaporthiopsis incrustans*	JF414846	499/539 (93)
RRFMV14–5	KJ855504	*Magnaporthiopsis incrustans*	JF414846	539/539 (100)
RS3–1	KJ855505	*Magnaporthiopsis incrustans*	JF414846	504/538 (94)
RS5–5	KJ855506	*Magnaporthiopsis incrustans*	JF414846	502/539 (93)
RS7–1	KJ855507	*Gaeumannomyces graminis* var. *graminis*	JF414846	536/539 (99)
RS7–2	KJ855508	*Magnaporthiopsis incrustans*	JF414846	500/537 (93)
RW3–4	KJ855509	Magnaporthales sp.	GQ923979	506/544 (91)
SPKBG7	KJ855510	*Magnaporthiopsis poae*	DQ528789	510/510 (100)
TAP35	KJ855511	*Magnaporthiopsis poae*	DQ528783	511/512 (99)
TAP41	KJ855512	*Magnaporthiopsis poae*	DQ528779	509/510 (99)
TPC4–5	KJ855513	*Magnaporthiopsis incrustans*	JF414846	501/539 (93)
TPC5–3	KJ855514	*Magnaporthiopsis incrustans*	JF414846	501/539 (93)
WW3–5	KJ855515	*Magnaporthiopsis incrustans*	JF414846	541/541 (100)

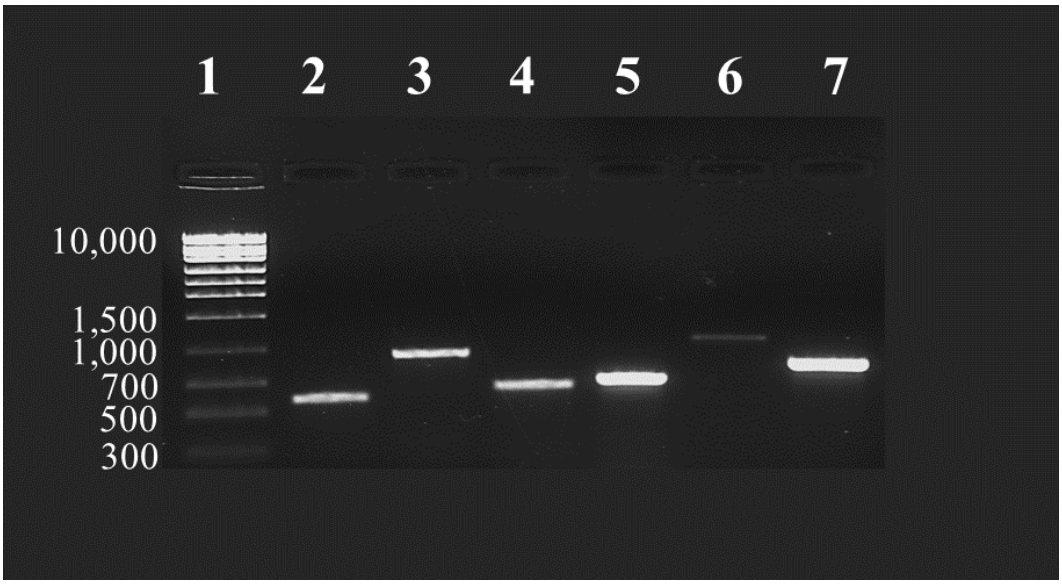


Figure B.7 Electrophoretic analysis of polymerase chain reaction amplification for phylogenetic markers of isolate GSGC15–4 on a 1.0% agarose gel.

Lane descriptions: 1 – 1kb DNA Ladder; 2 – approximately 550bp ITS product; 3 – approximately 870bp LSU product; 4 – approximately 580bp SSU product; 5 – approximately 620bp *MCM7* product; 6 – approximately 920bp *TEF1* product; 7 – approximately 700bp product.

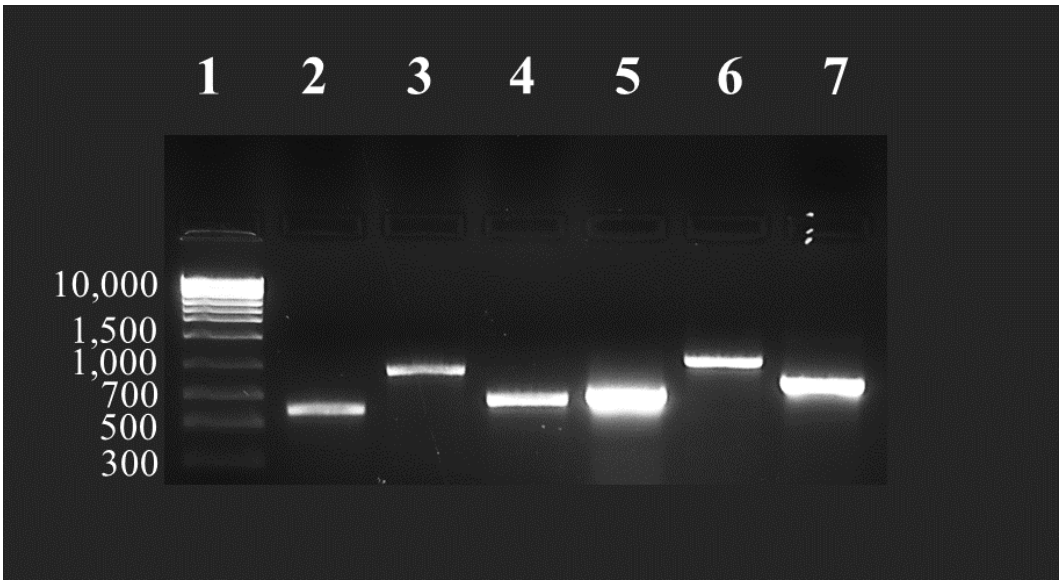


Figure B.8 Electrophoretic analysis of polymerase chain reaction amplification for phylogenetic markers of isolate DR1–4 on a 1.0% agarose gel.

Lane descriptions: 1 – 1kb DNA Ladder; 2 – approximately 550bp ITS product; 3 – approximately 870bp LSU product; 4 – approximately 580bp SSU product; 5 – approximately 620bp *MCM7* product; 6 – approximately 920bp *TEF1* product; 7 – approximately 700bp product.

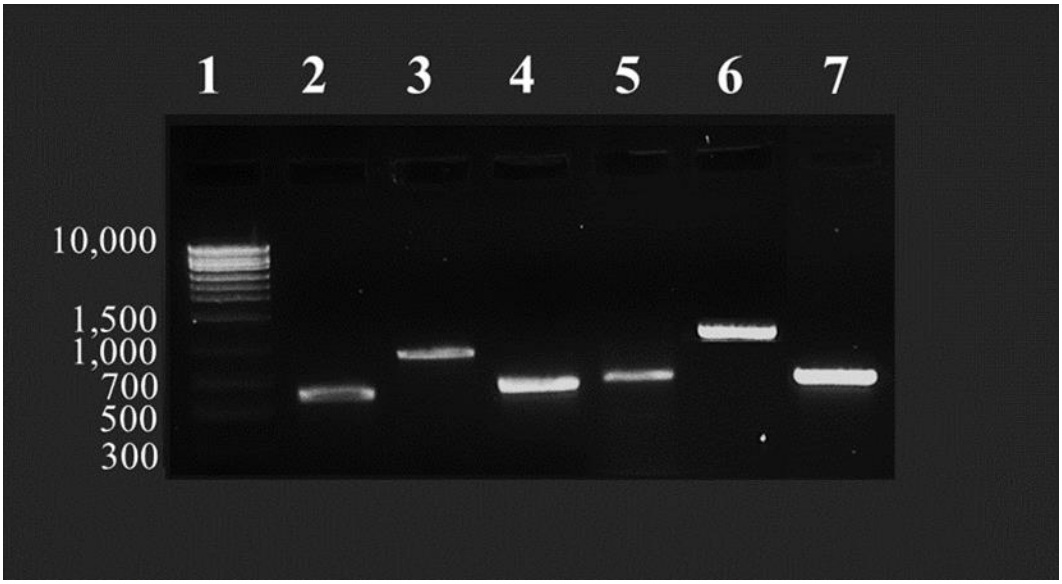


Figure B.9 Electrophoretic analysis of polymerase chain reaction amplification for phylogenetic markers of isolate WW3–5 on a 1.0% agarose gel.

Lane descriptions: 1 – 1kb DNA Ladder; 2 – approximately 550bp ITS product; 3 – approximately 870bp LSU product; 4 – approximately 580bp SSU product; 5 – approximately 620bp *MCM7* product; 6 – approximately 920bp *TEF1* product; 7 – approximately 700bp product.

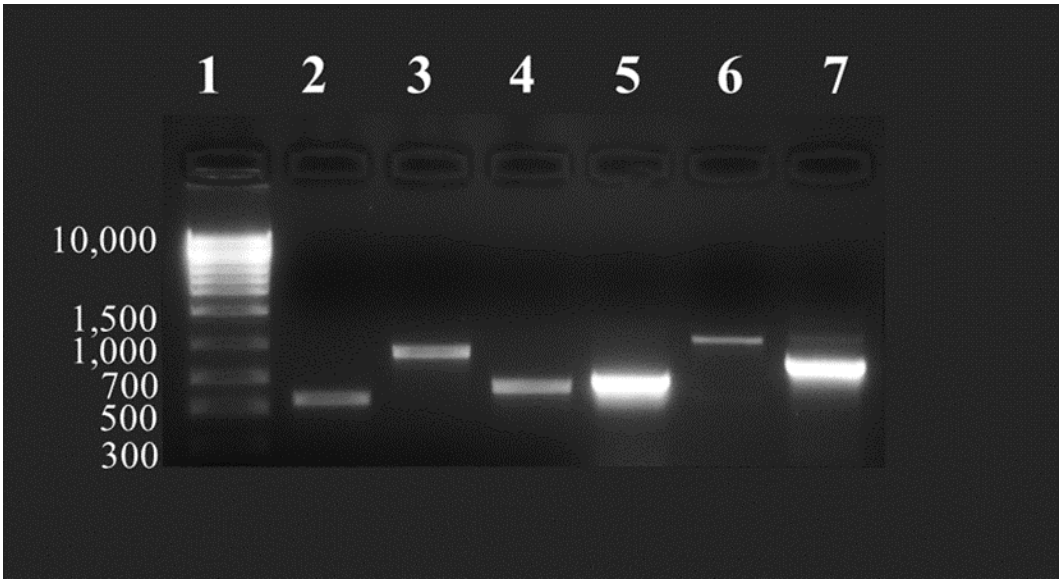


Figure B.10 Electrophoretic analysis of polymerase chain reaction amplification for phylogenetic markers of isolate KR10–6 on a 1.0% agarose gel.

Lane descriptions: 1 – 1kb DNA Ladder; 2 – approximately 550bp ITS product; 3 – approximately 870bp LSU product; 4 – approximately 580bp SSU product; 5 – approximately 620bp *MCM7* product; 6 – approximately 920bp *TEF1* product; 7 – approximately 700bp product.

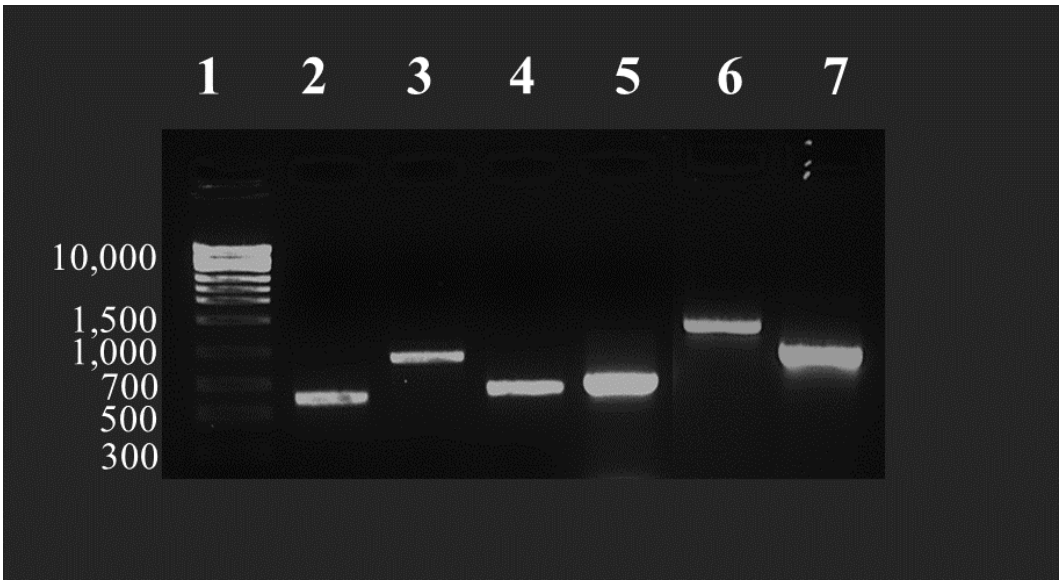


Figure B.11 Electrophoretic analysis of polymerase chain reaction amplification for phylogenetic markers of isolate RS7–2 on a 1.0% agarose gel.

Lane descriptions: 1 – 1kb DNA Ladder; 2 – approximately 550bp ITS product; 3 – approximately 870bp LSU product; 4 – approximately 580bp SSU product; 5 – approximately 620bp *MCM7* product; 6 – approximately 920bp *TEF1* product; 7 – approximately 700bp product.

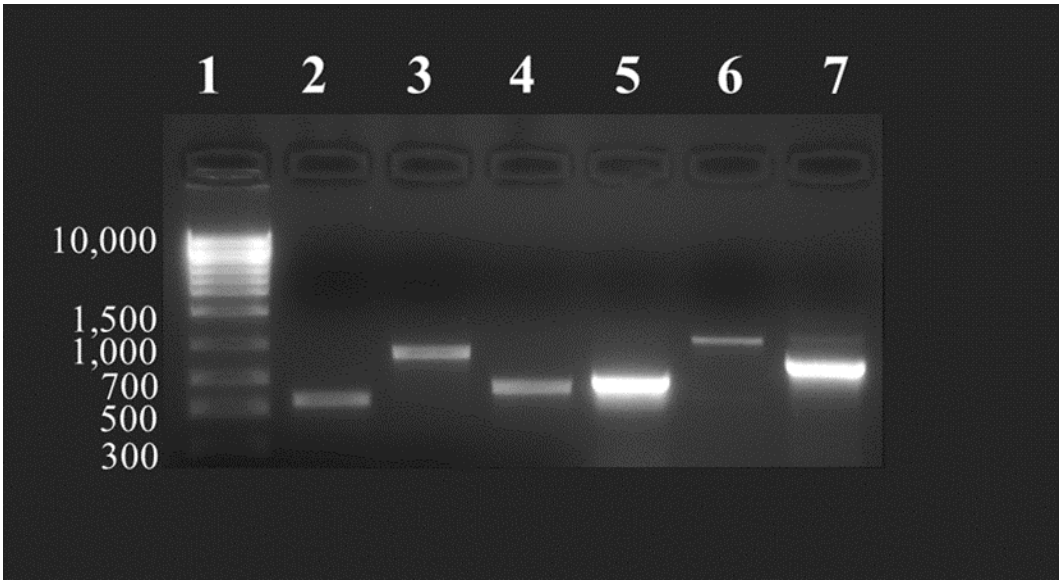


Figure B.12 Electrophoretic analysis of polymerase chain reaction amplification for phylogenetic markers of isolate RRFCHMP1–3 on a 1.0% agarose gel.

Lane descriptions: 1 – 1kb DNA Ladder; 2 – approximately 550bp ITS product; 3 – approximately 870bp LSU product; 4 – approximately 580bp SSU product; 5 – approximately 620bp *MCM7* product; 6 – approximately 920bp *TEF1* product; 7 – approximately 700bp product.

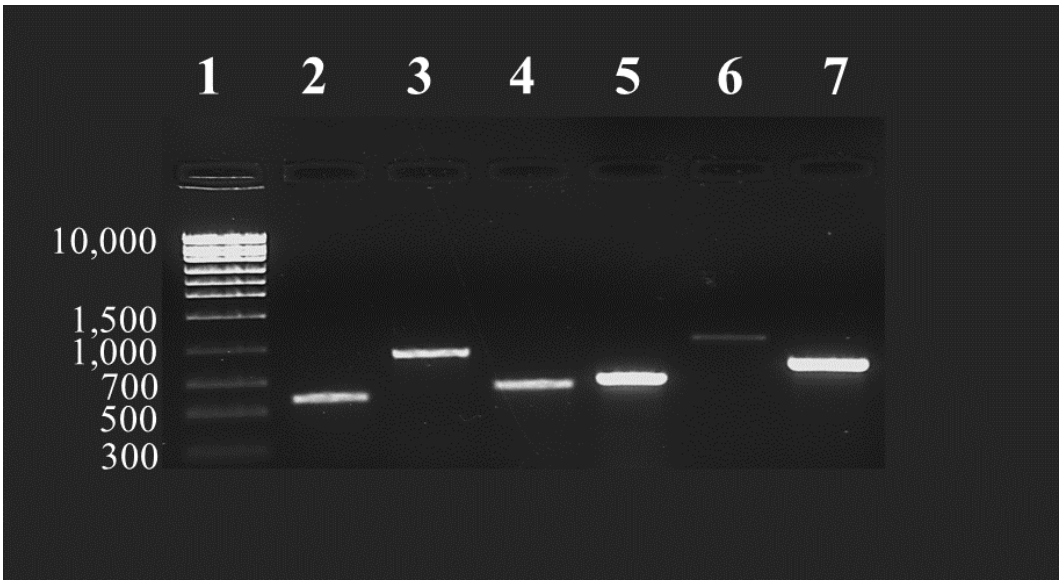


Figure B.13 Electrophoretic analysis of polymerase chain reaction amplification for phylogenetic markers of isolate HP24–3 on a 1.0% agarose gel.

Lane descriptions: 1 – 1kb DNA Ladder; 2 – approximately 550bp ITS product; 3 – approximately 870bp LSU product; 4 – approximately 580bp SSU product; 5 – approximately 620bp *MCM7* product; 6 – approximately 920bp *TEF1* product; 7 – approximately 700bp product.

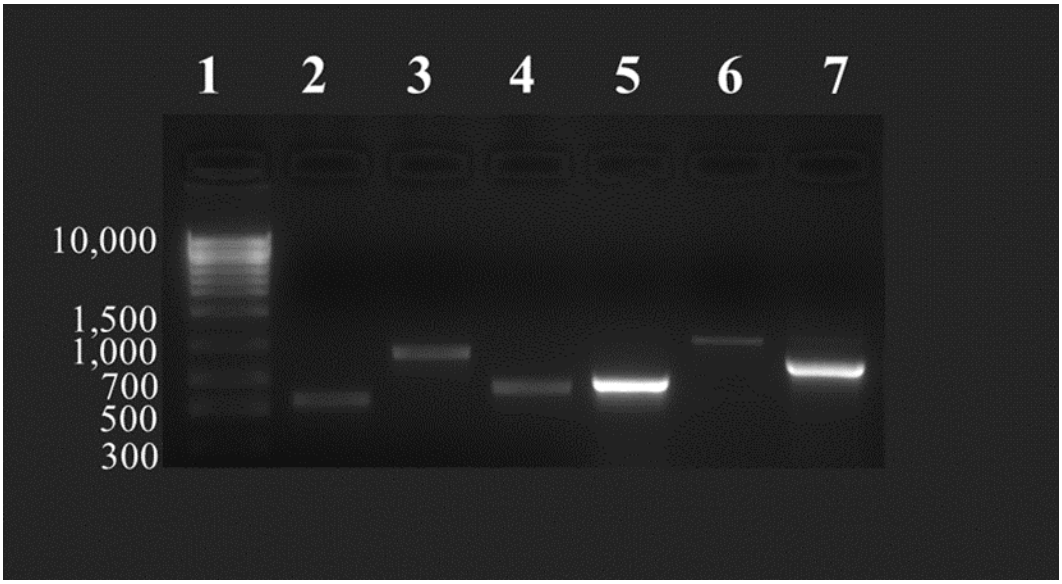


Figure B.14 Electrophoretic analysis of polymerase chain reaction amplification for phylogenetic markers of isolate RW3–4 on a 1.0% agarose gel.

Lane descriptions: 1 – 1kb DNA Ladder; 2 – approximately 550bp ITS product; 3 – approximately 870bp LSU product; 4 – approximately 580bp SSU product; 5 – approximately 620bp *MCM7* product; 6 – approximately 920bp *TEF1* product; 7 – approximately 700bp product.

Figure B.15 Incubation chambers set to 18, 25, 32, and 38 C for optimal growing temperature studies of ectotrophic root-infecting fungi.

Figure B.16 Placement of ERI fungal isolates in incubation chambers for optimal growing temperature studies.

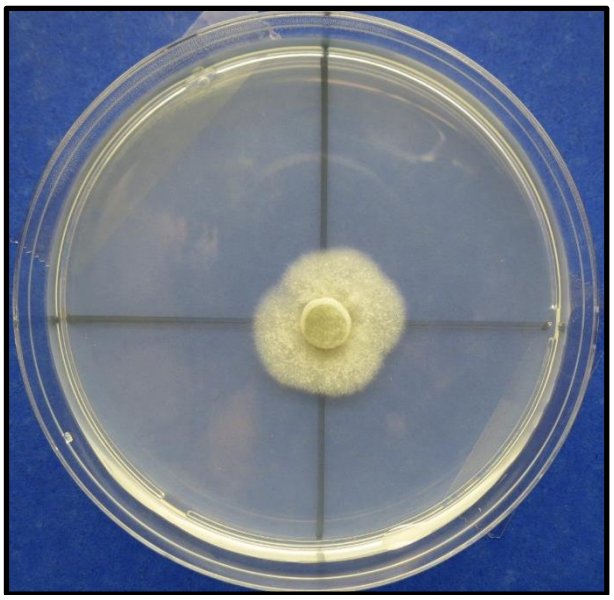


Figure B.17 Mycelial growth ERI fungal isolate after 7-day incubation at 18 C.

Figure B.18 Mycelial growth of ERI fungal isolate after 7-day incubation at 25 C.

Figure B.19 Mycelial growth of ERI fungal isolate after 7-day incubation at 32 C.

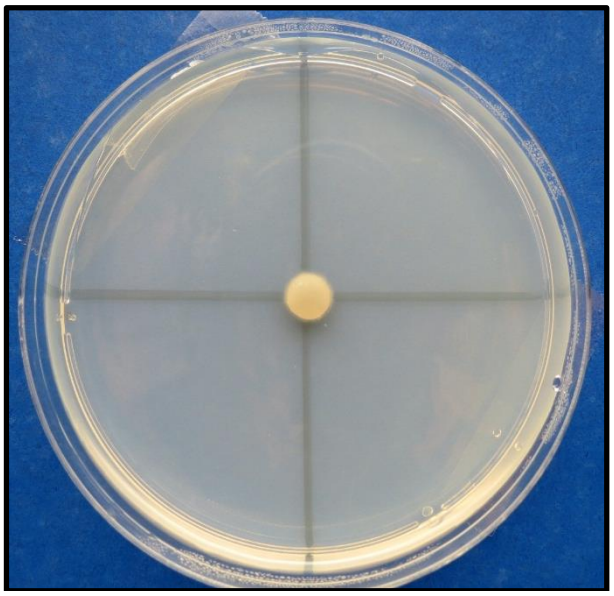


Figure B.20 Mycelial growth of ERI fungal isolate after 7-day incubation at 38 C.

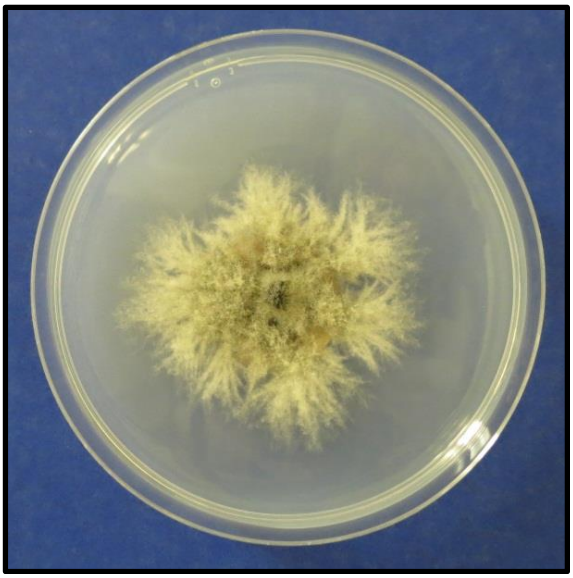


Figure B.21 Colony morphology of *Gaeumannomyces graminis* var. *graminis* isolate AD1–2 at 7-day maturity on potato dextrose agar.

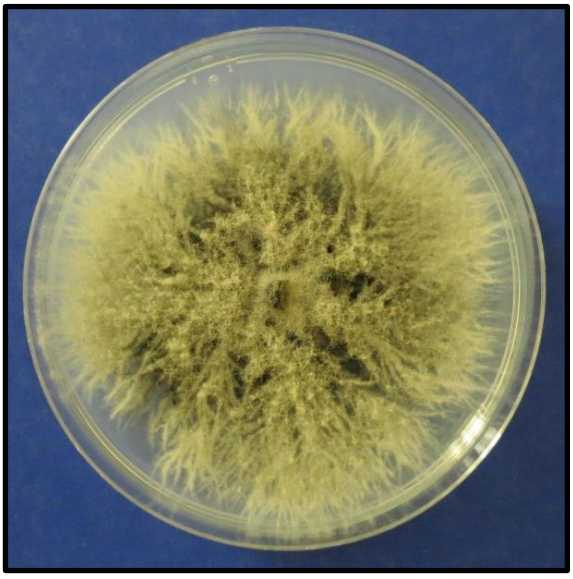


Figure B.22 Colony morphology of *Gaeumannomyces graminis* var. *graminis* isolate AD1–2 at 10-day maturity on potato dextrose agar.

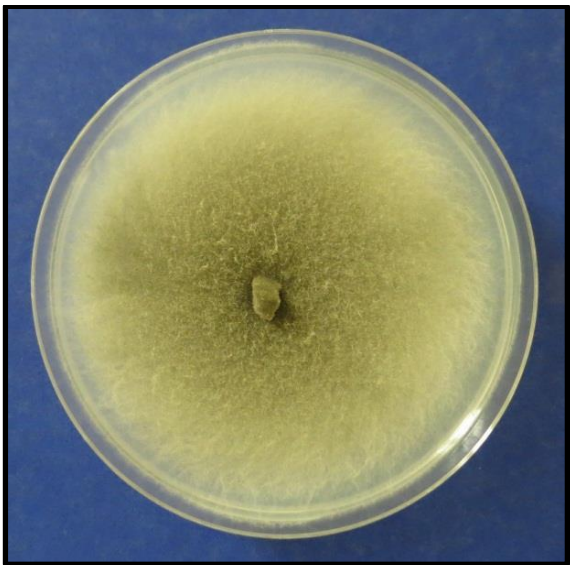


Figure B.23 Colony morphology of *Gaeumannomyces paulograminis* isolate DR10–3 at 7-day maturity on potato dextrose agar.

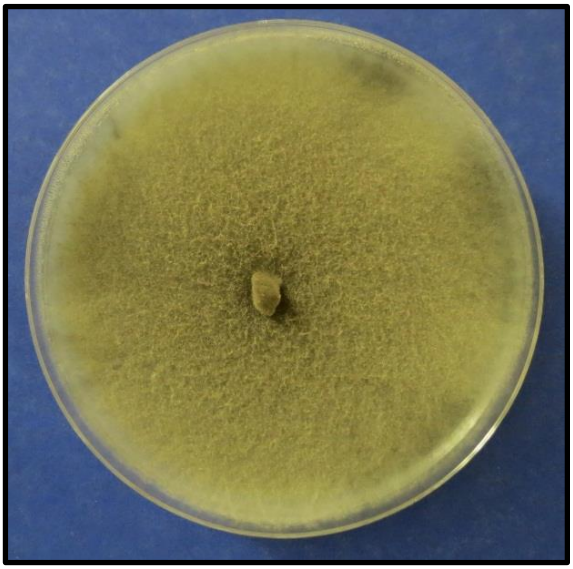


Figure B.24 Colony morphology of *Gaeumannomyces paulograminis* isolate DR10–3 at 10-day maturity on potato dextrose agar.

Figure B.25 Colony morphology of *Gaeumannomyces paulograminis* isolate DR12–1 at 7-day maturity on potato dextrose agar.

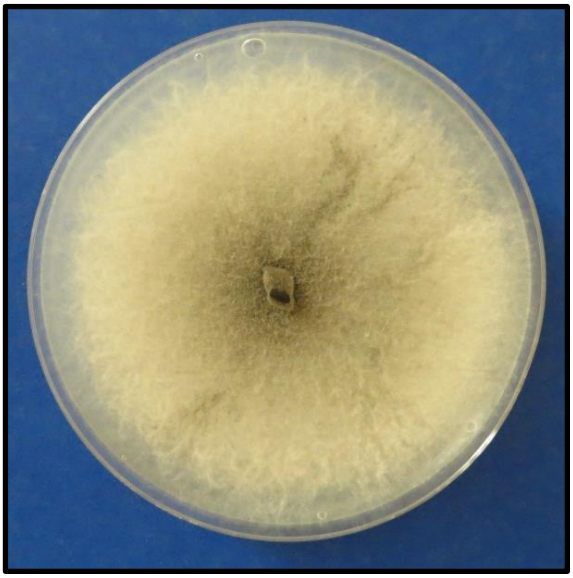


Figure B.26 Colony morphology of *Gaeumannomyces paulograminis* isolate DR12–1 at 10-day maturity on potato dextrose agar.

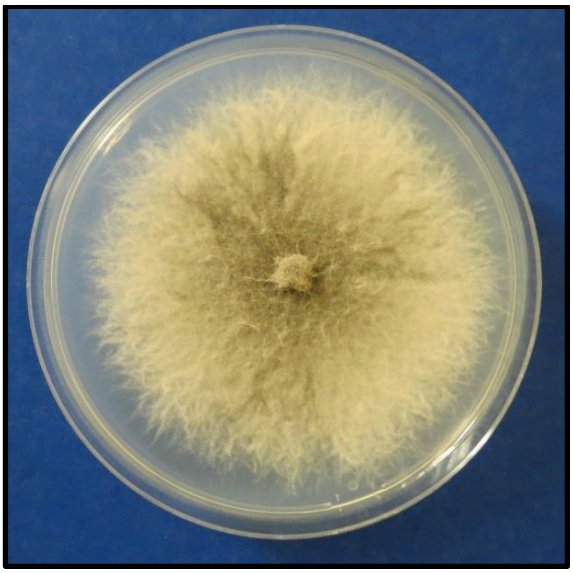


Figure B.27 Colony morphology of *Gaeumannomyces paulograminis* isolate DR13–1 at 7-day maturity on potato dextrose agar.

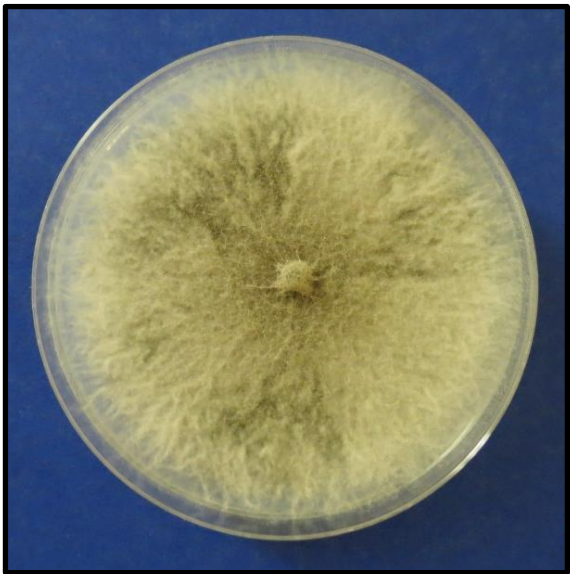


Figure B.28 Colony morphology of *Gaeumannomyces paulograminis* isolate DR13–1 at 10-day maturity on potato dextrose agar.

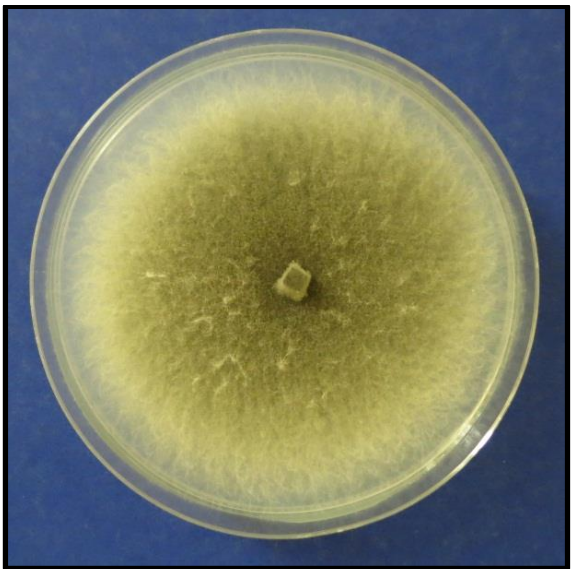


Figure B.29 Colony morphology of *Gaeumannomyces paulograminis* isolate DR1–4 at 7-day maturity on potato dextrose agar.

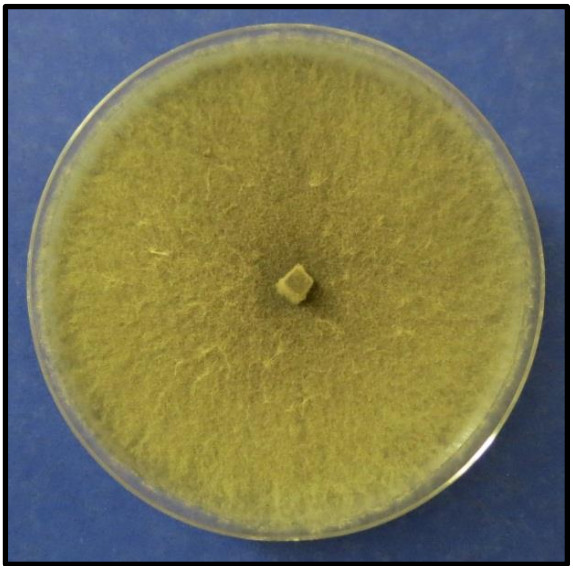


Figure B.30 Colony morphology of *Gaeumannomyces paulograminis* isolate DR1–4 at 10-day maturity on potato dextrose agar.

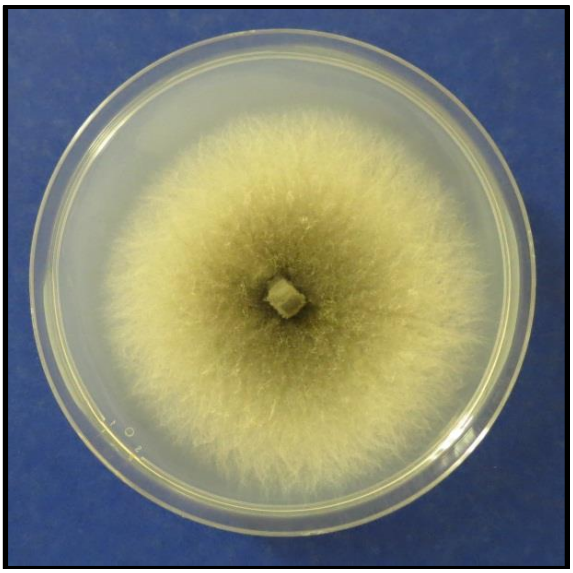


Figure B.31 Colony morphology of *Gaeumannomyces paulograminis* isolate DR6–4 at 7-day maturity on potato dextrose agar.

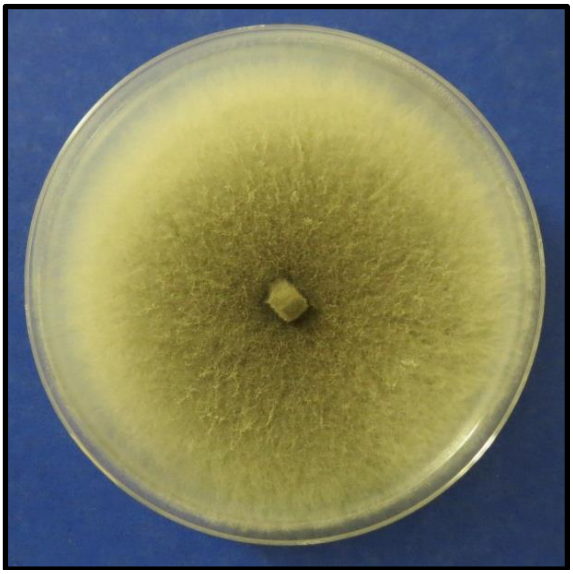


Figure B.32 Colony morphology of *Gaeumannomyces paulograminis* isolate DR6–4 at 10-day maturity on potato dextrose agar.

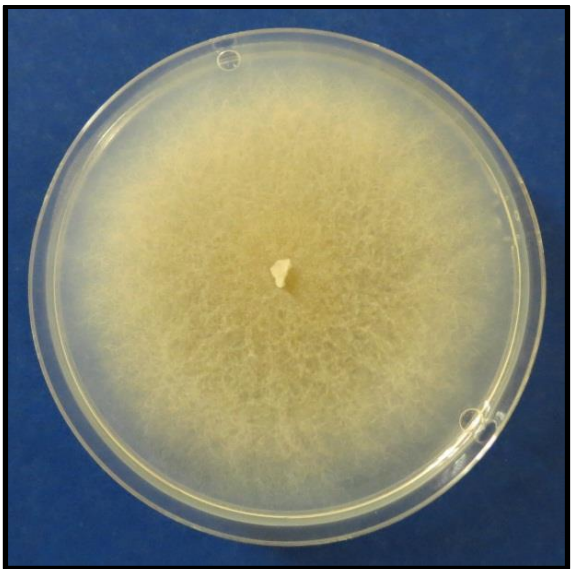


Figure B.33 Colony morphology of *Magnaporthiopsis taurocanis* isolate GSGC10–2 at 7-day maturity on potato dextrose agar.

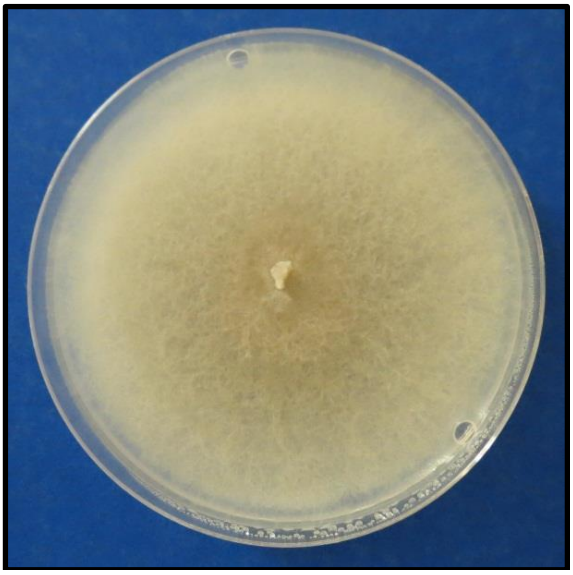


Figure B.34 Colony morphology of *Magnaporthiopsis taurocanis* isolate GSGC10–2 at 10-day maturity on potato dextrose agar.

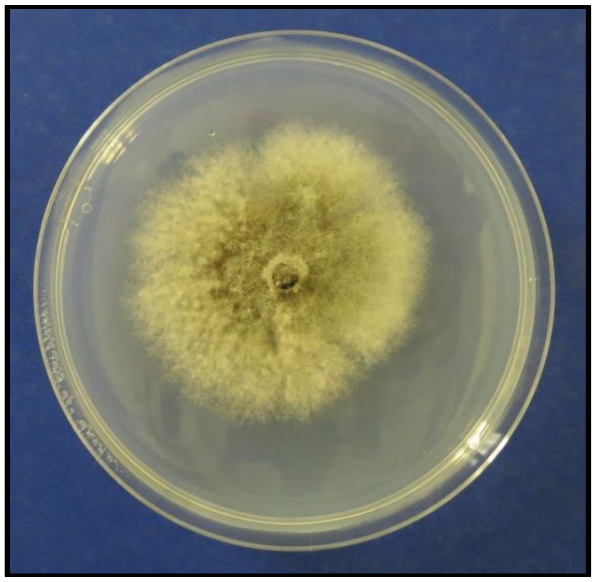


Figure B.35 Colony morphology of *Gaeumannomyces graminis* var. *graminis* isolate GSGC15–3 at 7-day maturity on potato dextrose agar.

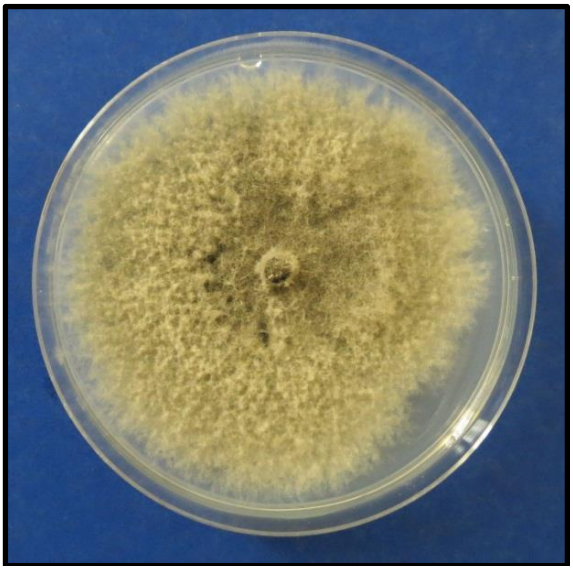


Figure B.36 Colony morphology of *Gaeumannomyces graminis* var. *graminis* isolate GSGC15–3 at 10-day maturity on potato dextrose agar.

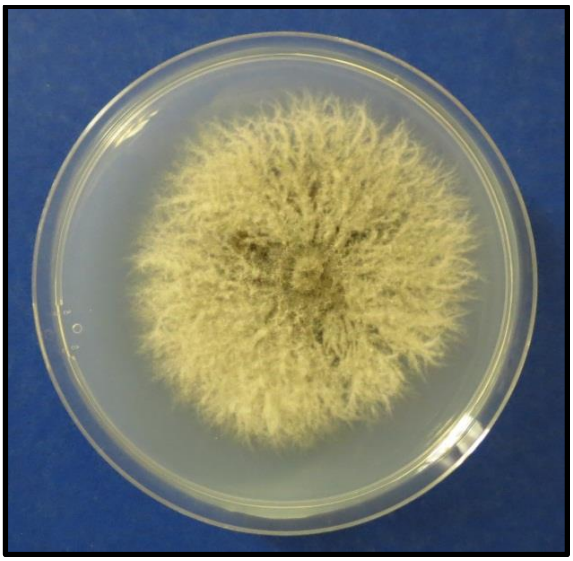


Figure B.37 Colony morphology of *Gaeumannomyces graminis* var. *graminis* isolate GSGC15–4 at 7-day maturity on potato dextrose agar.

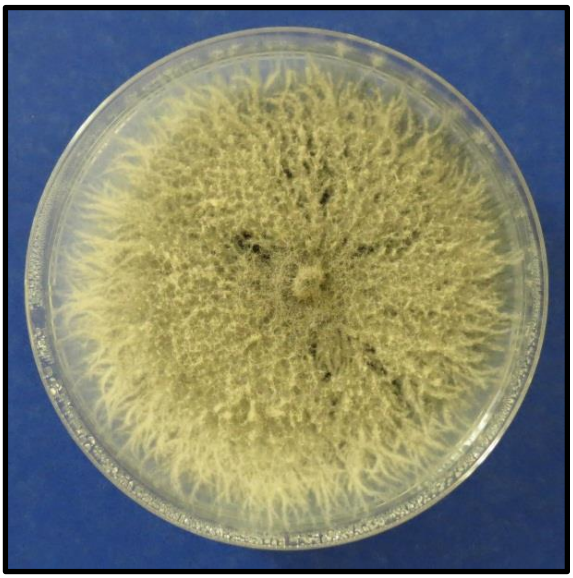


Figure B.38 Colony morphology of *Gaeumannomyces graminis* var. *graminis* isolate GSGC15–4 at 10-day maturity on potato dextrose agar.

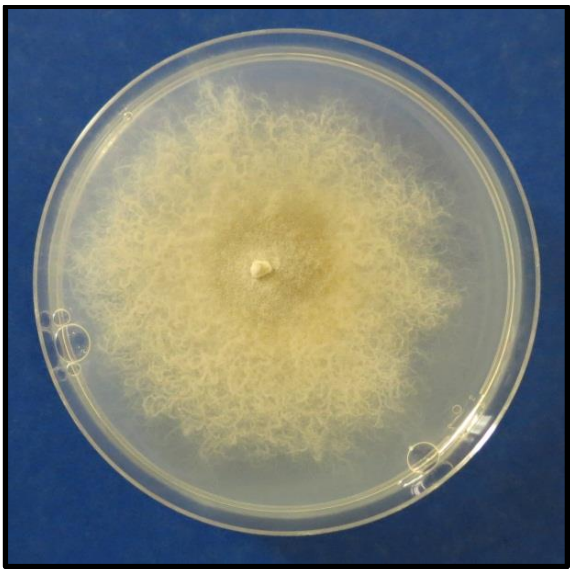


Figure B.39 Colony morphology of *Magnaporthiopsis cynodontis* isolate HCC3–4 at 7-day maturity on potato dextrose agar.

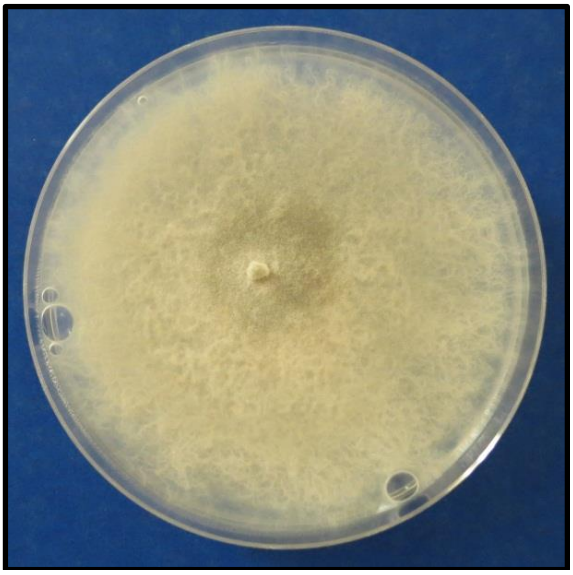


Figure B.40 Colony morphology of *Magnaporthiopsis cynodontis* isolate HCC3–4 at 10-day maturity on potato dextrose agar.

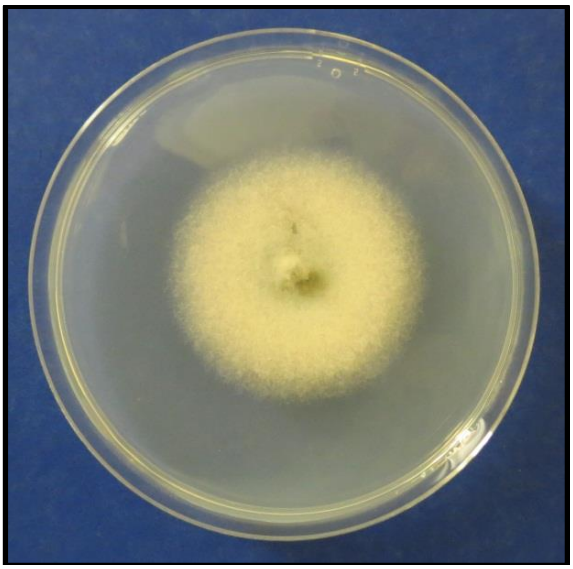


Figure B.41 Colony morphology of *Candidacolonium cynodontis* isolate HP24–3 at 7-day maturity on potato dextrose agar.

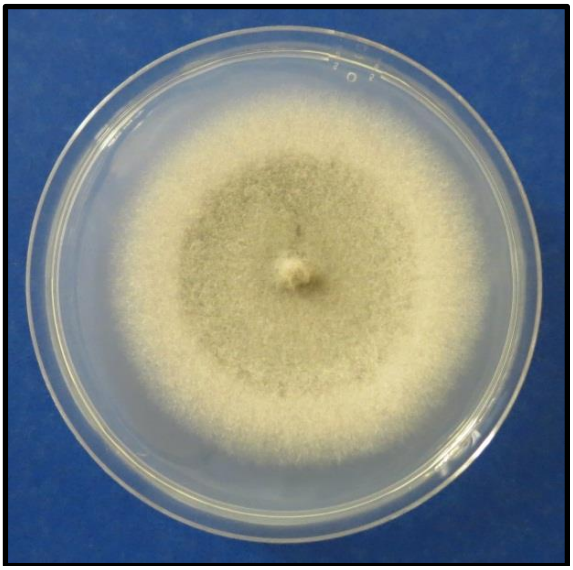


Figure B.42 Colony morphology of *Candidacolonium cynodontis* isolate HP24–3 at 10-day maturity on potato dextrose agar.

Figure B.43 Colony morphology of *Candidacolonium cynodontis* isolate HP38–4 at 7-day maturity on potato dextrose agar.

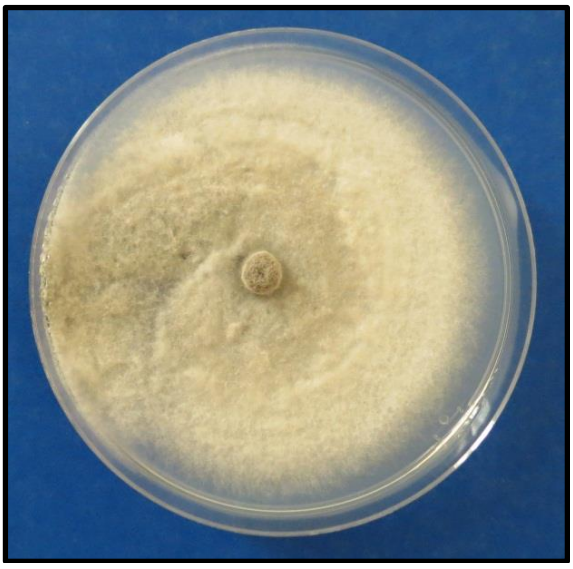


Figure B.44 Colony morphology of *Candidacolonium cynodontis* isolate HP38–4 at 10-day maturity on potato dextrose agar.

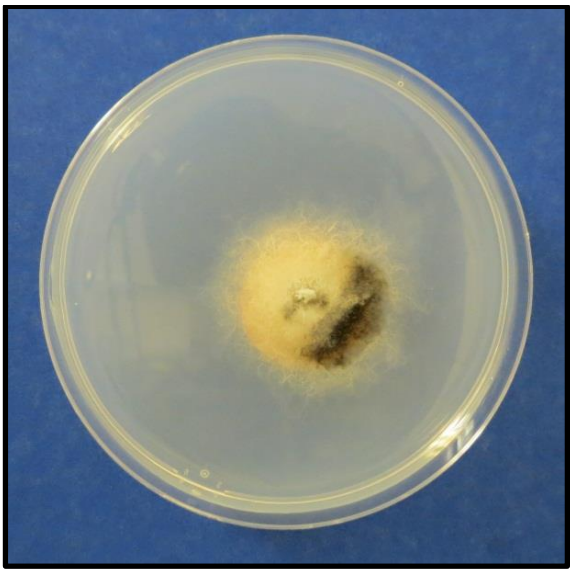


Figure B.45 Colony morphology of *Magnaporthiopsis hawaiiensis* isolate KR10–6 at 7-day maturity on potato dextrose agar.

Figure B.46 Colony morphology of *Magnaporthiopsis hawaiiensis* isolate KR10–6 at 10-day maturity on potato dextrose agar.

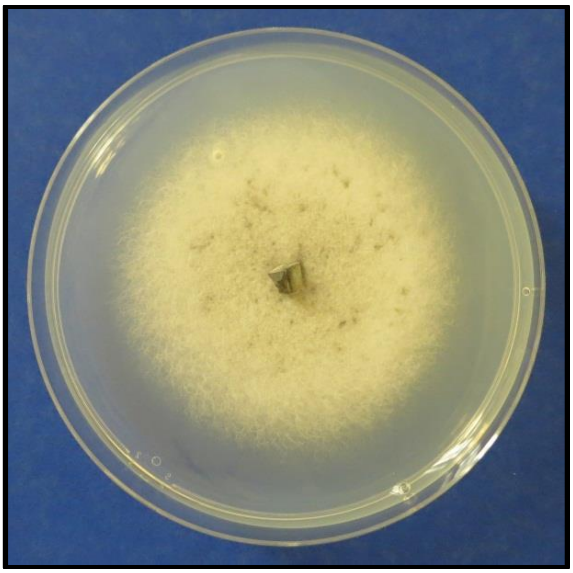


Figure B.47 Colony morphology of *Magnaporthiopsis incrustans* isolate LC8–6 at 7-day maturity on potato dextrose agar.

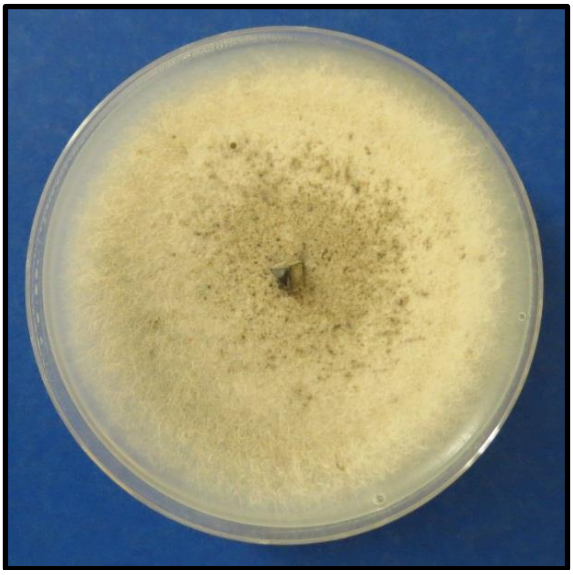


Figure B.48 Colony morphology of *Magnaporthiopsis incrustans* isolate LC8–6 at 10-day maturity on potato dextrose agar.

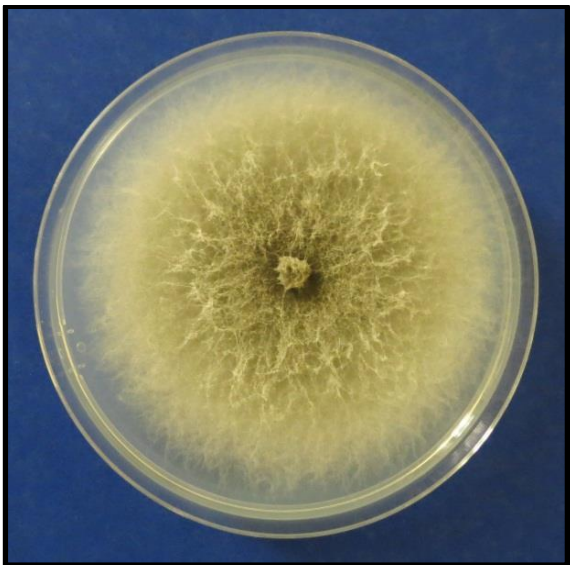


Figure B.49 Colony morphology of *Gaeumannomyces paulograminis* isolate OW4–4 at 7-day maturity on potato dextrose agar.

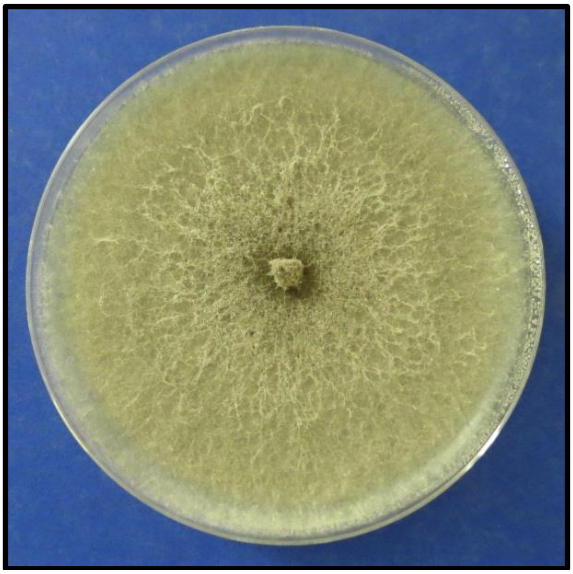


Figure B.50 Colony morphology of *Gaeumannomyces paulograminis* isolate OW4–4 at 10-day maturity on potato dextrose agar.

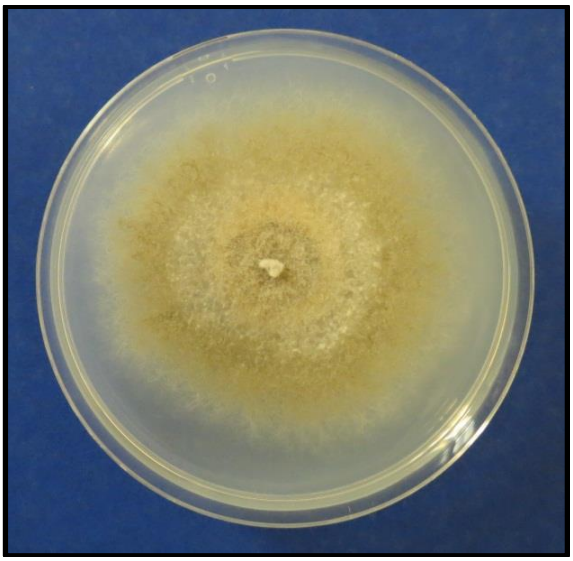


Figure B.51 Colony morphology of *Magnaporthiopsis taurocanis* isolate RRFCHMP1–3 at 7-day maturity on potato dextrose agar.

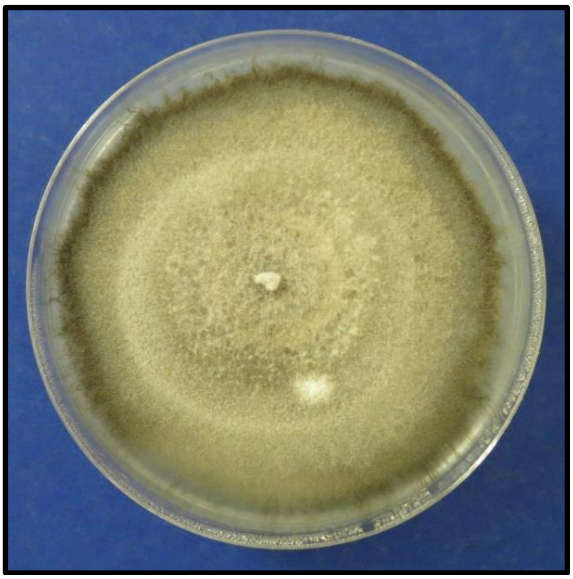


Figure B.52 Colony morphology of *Magnaporthiopsis taurocanis* isolate RRFCHMP1–3 at 10-day maturity on potato dextrose agar.

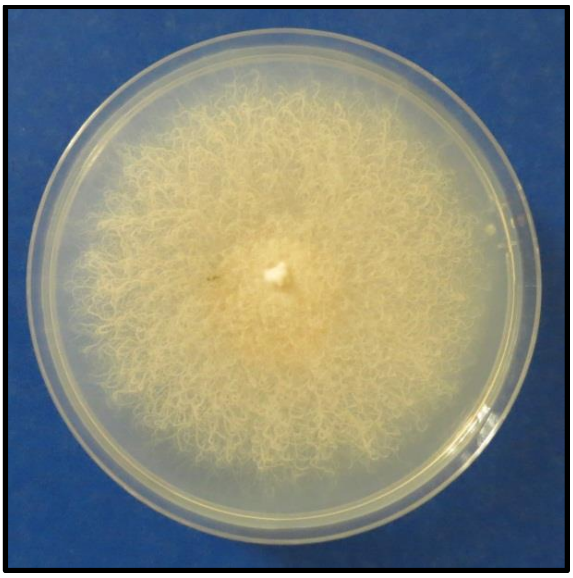


Figure B.53 Colony morphology of *Magnaporthiopsis taurocanis* isolate RRFMV10–2 at 7-day maturity on potato dextrose agar.

Figure B.54 Colony morphology of *Magnaporthiopsis taurocanis* isolate RRFMV10–2 at 10-day maturity on potato dextrose agar.

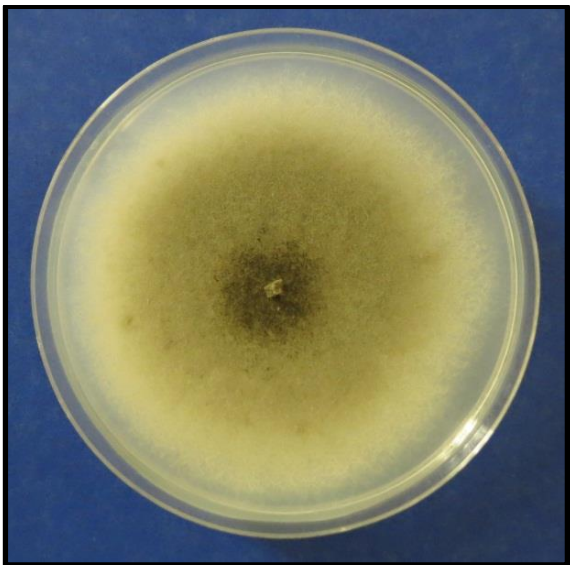


Figure B.55 Colony morphology of *Magnaporthiopsis incrustans* isolate RRFMV14–5 at 7-day maturity on potato dextrose agar.

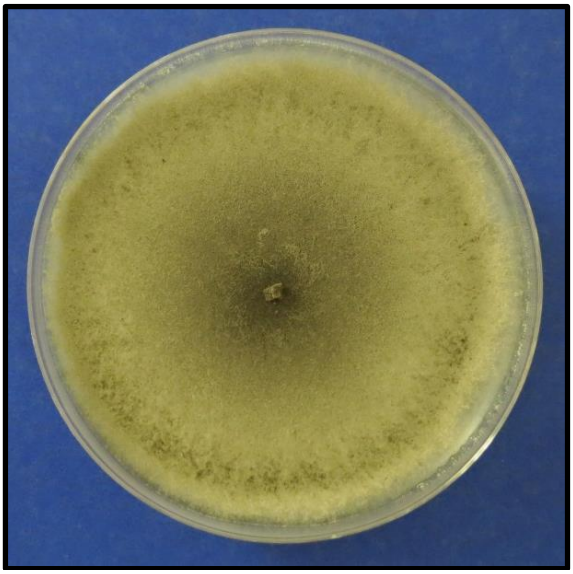


Figure B.56 Colony morphology of *Magnaporthiopsis incrustans* isolate RRFMV14–5 at 10-day maturity on potato dextrose agar.

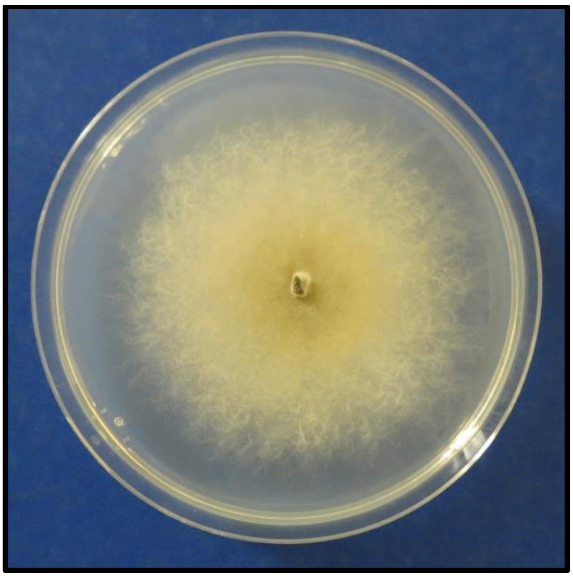


Figure B.57 Colony morphology of *Magnaporthiopsis cynodontis* isolate RS3–1 at 7-day maturity on potato dextrose agar.

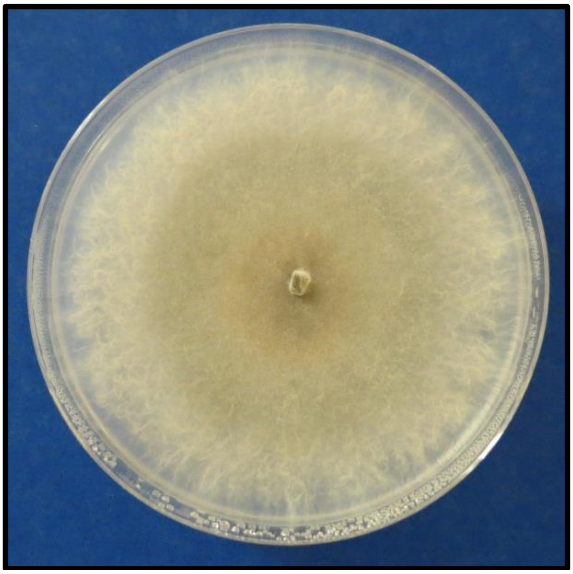


Figure B.58 Colony morphology of *Magnaporthiopsis cynodontis* isolate RS3–1 at 10-day maturity on potato dextrose agar.

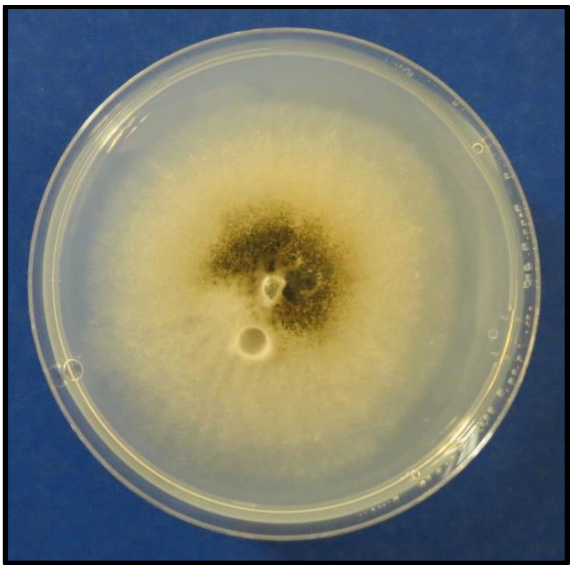


Figure B.59 Colony morphology of *Magnaporthiopsis cynodontis* isolate RS5–5 at 7-day maturity on potato dextrose agar.

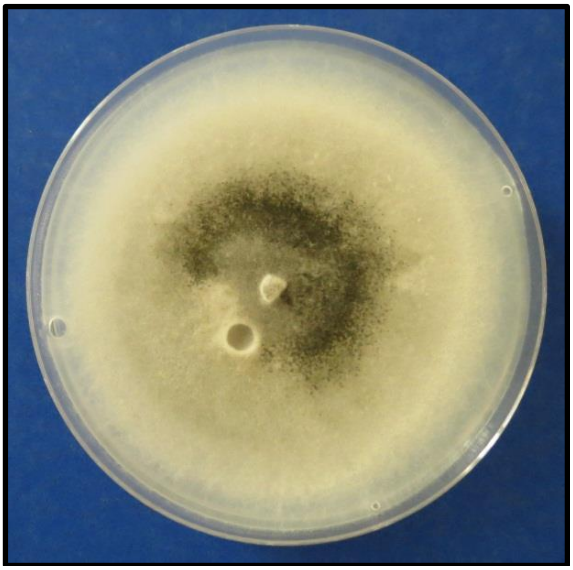


Figure B.60 Colony morphology of *Magnaporthiopsis cynodontis* isolate RS5–5 at 10-day maturity on potato dextrose agar.

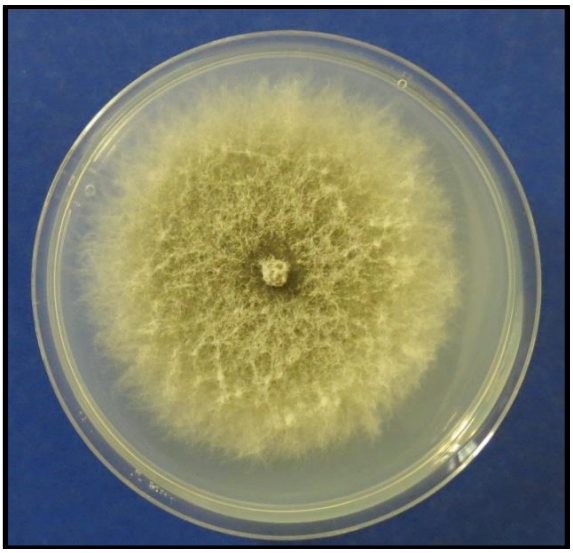


Figure B.61 Colony morphology of *Gaeumannomyces paulograminis* isolate RS7–1 at 7-day maturity on potato dextrose agar.

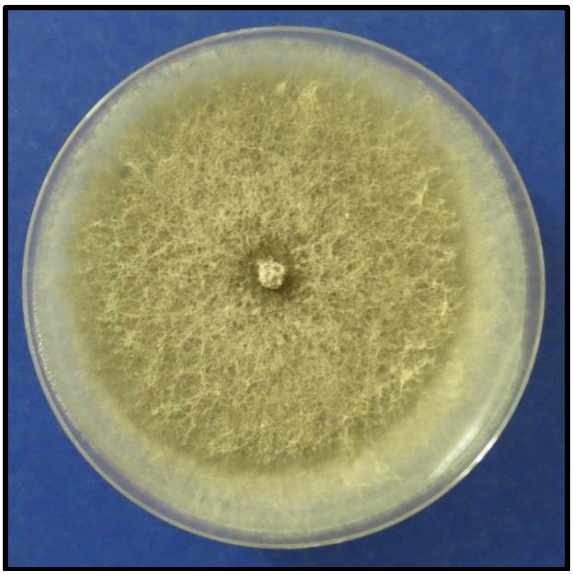


Figure B.62 Colony morphology of *Gaeumannomyces paulograminis* isolate RS7–1 at 10-day maturity on potato dextrose agar.

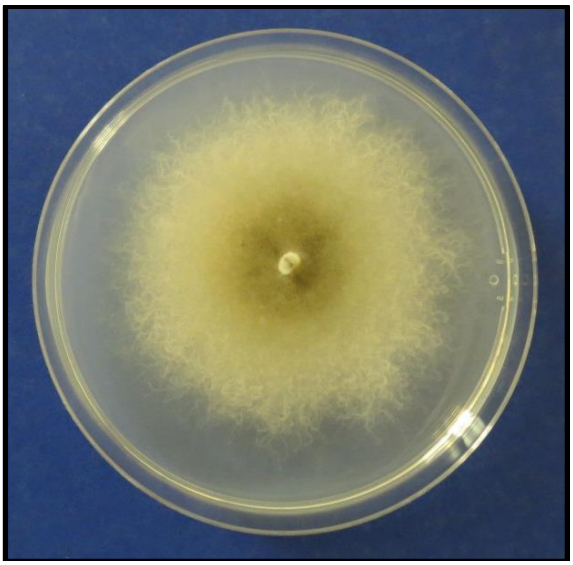


Figure B.63 Colony morphology of *Magnaporthiopsis cynodontis* isolate RS7–2 at 7-day maturity on potato dextrose agar.

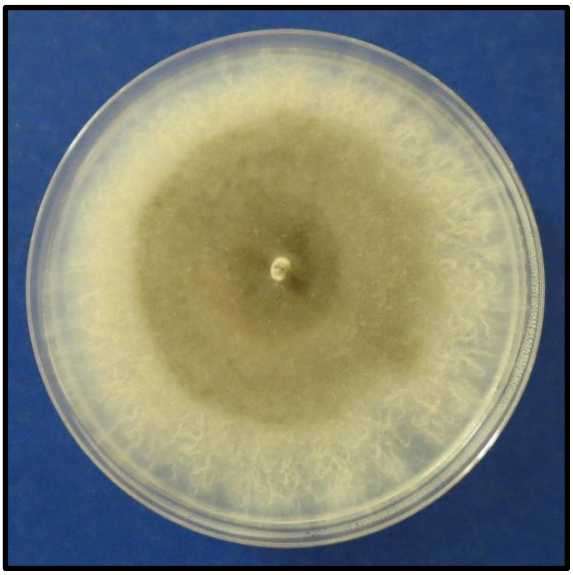


Figure B.64 Colony morphology of *Magnaporthiopsis cynodontis* isolate RS7–2 at 10-day maturity on potato dextrose agar.

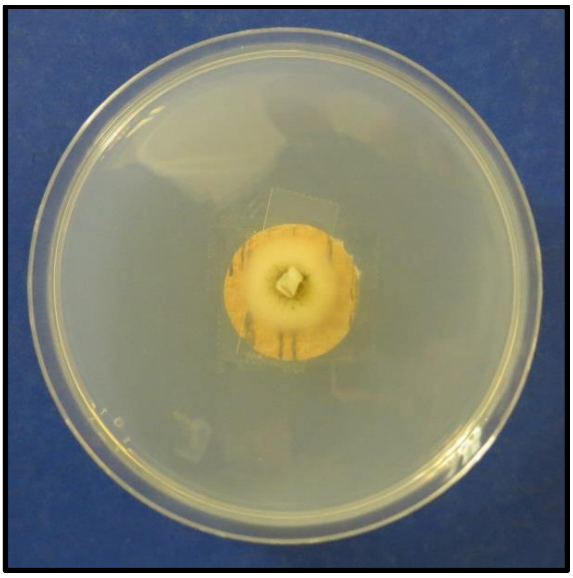


Figure B.65 Colony morphology of *Pseudophialophora cynodontis* isolate RW3–4 at 7-day maturity on potato dextrose agar.

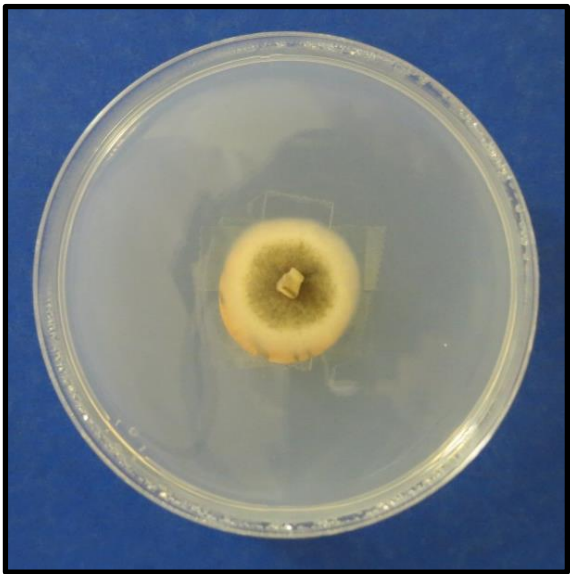


Figure B.66 Colony morphology of *Pseudophialophora cynodontis* isolate RW3–4 at 10-day maturity on potato dextrose agar.

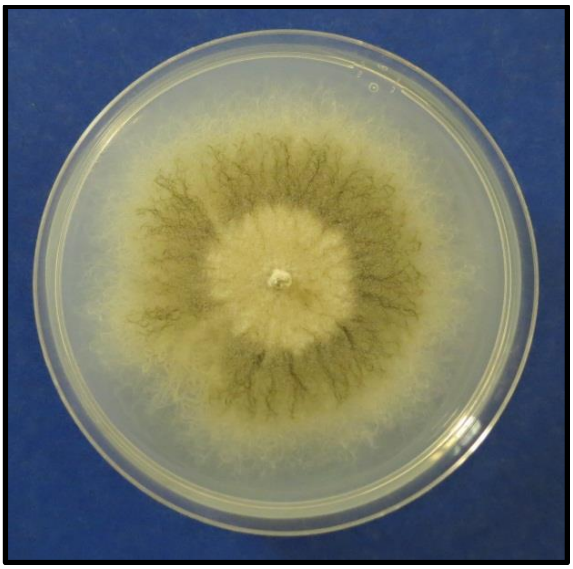


Figure B.67 Colony morphology of *Magnaporthiopsis taurocanis* isolate TPC4–5 at 7-day maturity on potato dextrose agar.

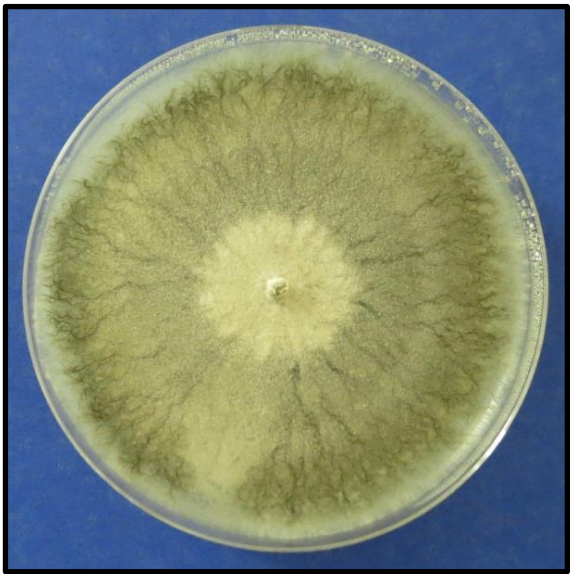


Figure B.68 Colony morphology of *Magnaporthiopsis taurocanis* isolate TPC4–5 at 10-day maturity on potato dextrose agar.

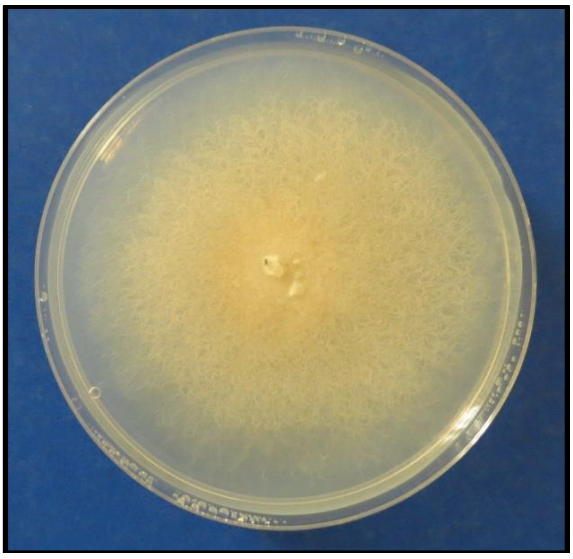


Figure B.69 Colony morphology of *Magnaporthiopsis taurocanis* isolate TPC5–3 at 7-day maturity on potato dextrose agar.

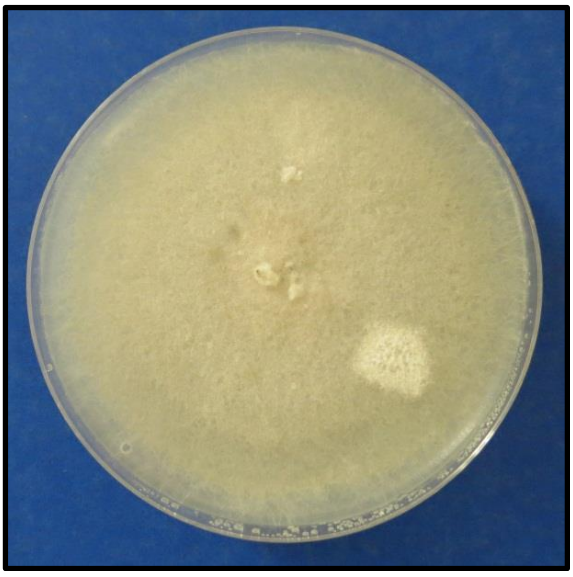


Figure B.70 Colony morphology of *Magnaporthiopsis taurocanis* isolate TPC5–3 at 10-day maturity on potato dextrose agar.

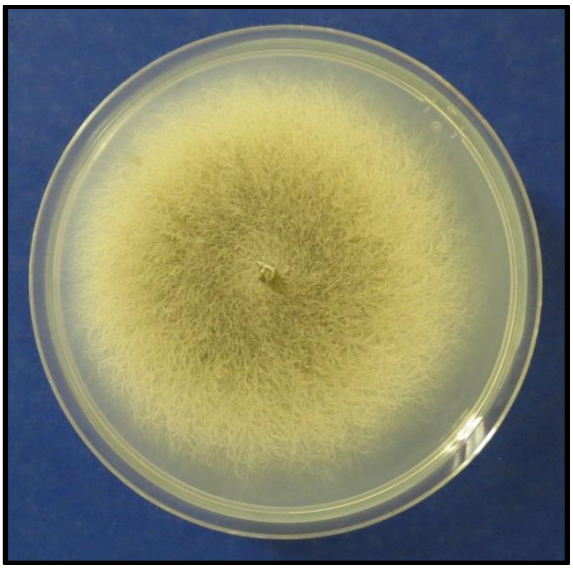


Figure B.71 Colony morphology of *Magnaporthiopsis incrustans* isolate WW3–5 at 7-day maturity on potato dextrose agar.

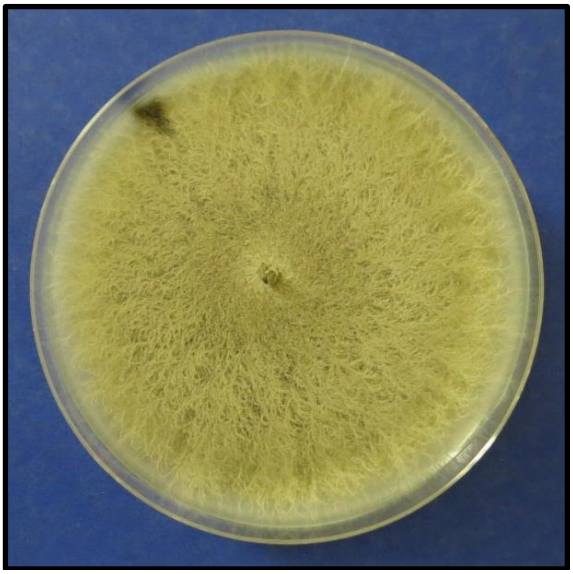


Figure B.72 Colony morphology of *Magnaporthiopsis incrustans* isolate WW3–5 at 10-day maturity on potato dextrose agar.

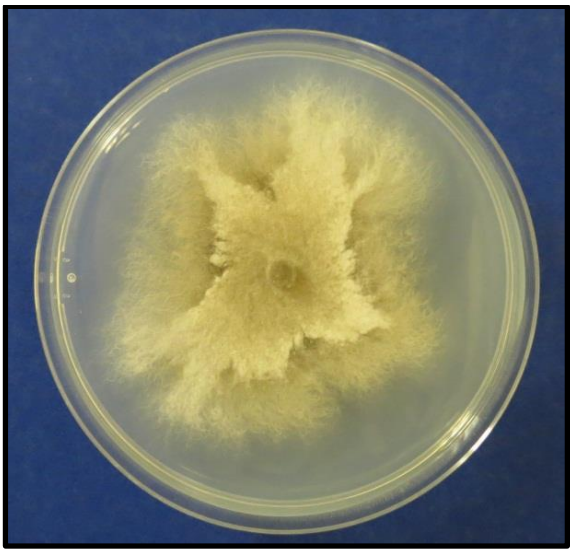


Figure B.73 Colony morphology of *Magnaporthiopsis poae* isolate TAP35 at 7-day maturity on potato dextrose agar.

Figure B.74 Colony morphology of *Magnaporthiopsis poae* isolate TAP35 at 10-day maturity on potato dextrose agar.

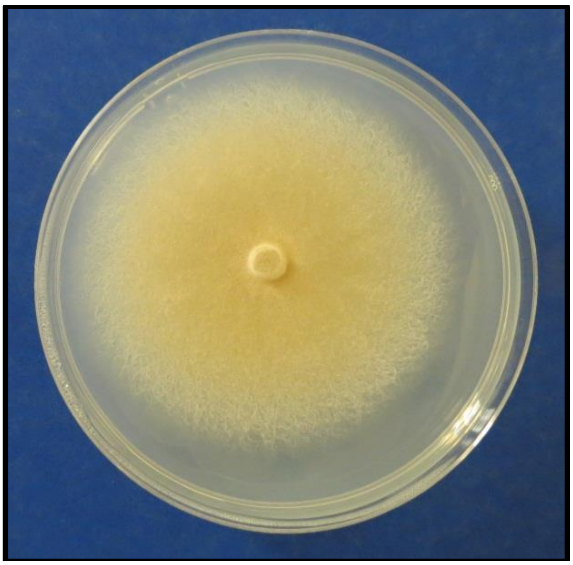


Figure B.75 Colony morphology of *Magnaporthiopsis poae* isolate TAP41 at 7-day maturity on potato dextrose agar.

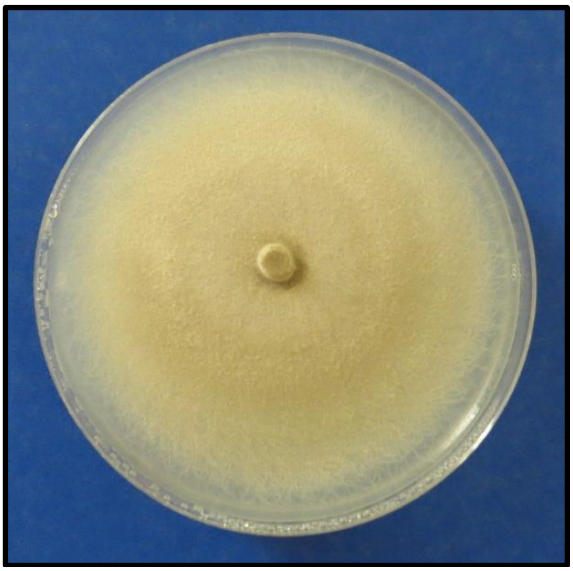


Figure B.76 Colony morphology of *Magnaporthiopsis poae* isolate TAP41 at 10-day maturity on potato dextrose agar.

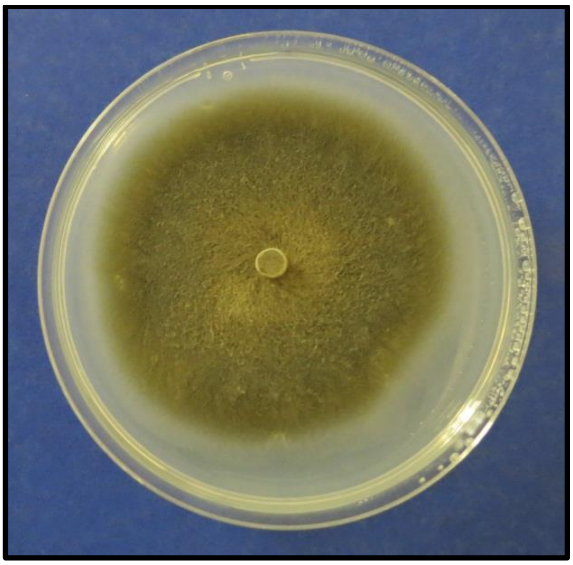


Figure B.77 Colony morphology of *Magnaporthiopsis poae* isolate SPKBG7 at 7-day maturity on potato dextrose agar.

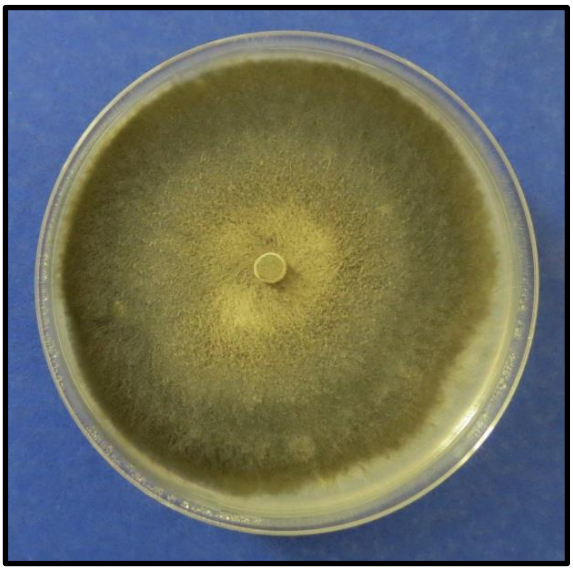


Figure B.78 Colony morphology of *Magnaporthiopsis poae* isolate SPKBG7 at 10-day maturity on potato dextrose agar.

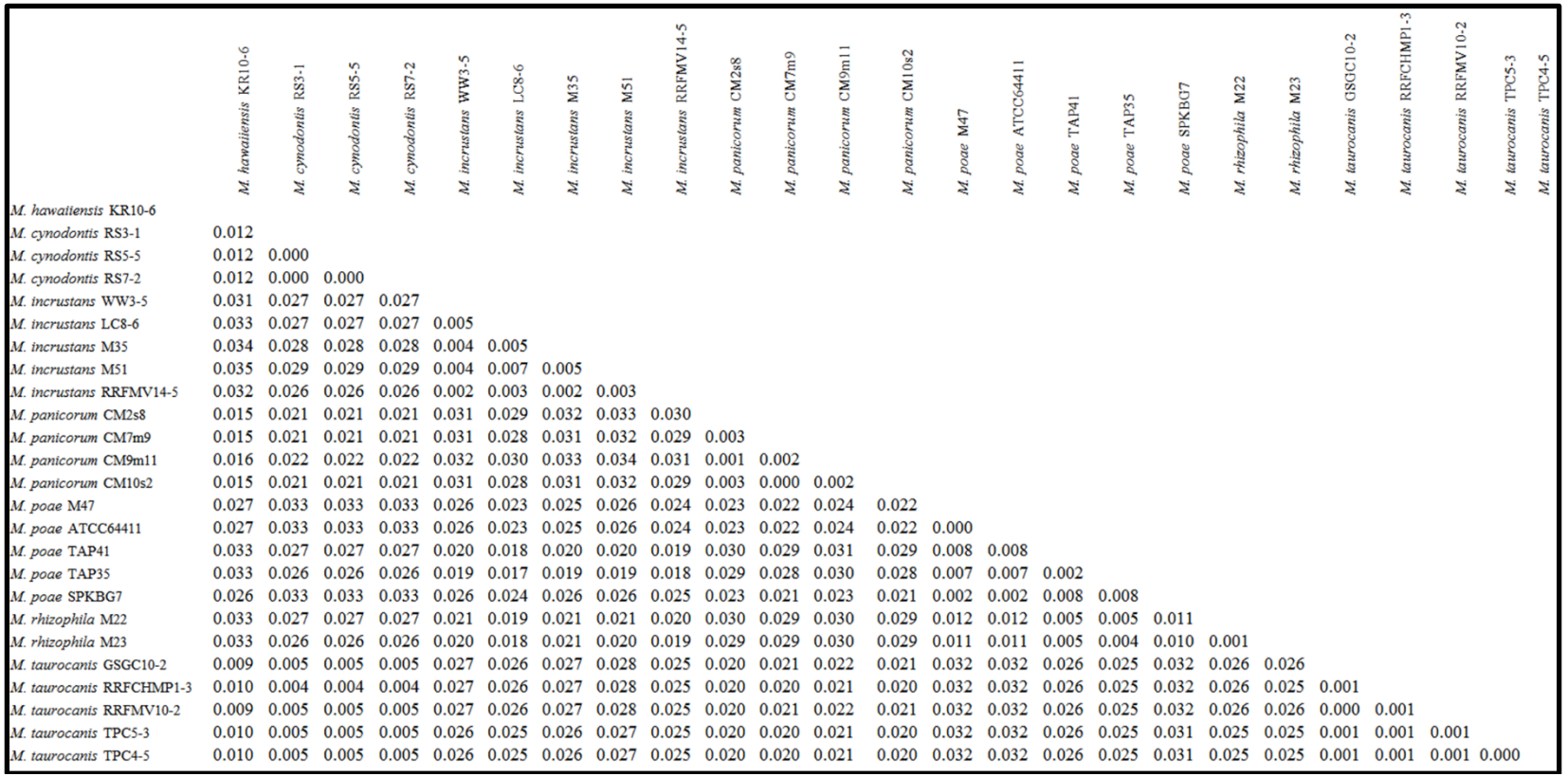
Table B.2 Maximum likelihood fits of nucleotide substitution models for the combined ITS, LSU, SSU, *MCM7*, *RPB1*, and *TEF1* dataset.

Model	**BIC**[†]
General Time Reversible (GTR) + G[§] + I[¶]	95739.31
GTR + G	95859.57
Tamura 3-parameter (T92) + G + I	95974.73
Kimura 2-parameter (K2) + G + I	96082.09
T92 + G	96093.19
K2 + G	96206.07
Hasegawa-Kishino-Yano (HKY) + G + I	96333.59
GTR + I	96546.95
T92 + I	96694.93
K2 + I	96747.85
Tamura-Nei (TN93) + I	96977.97
Jukes-Cantor (JC) + G + I	97023.29
HKY + I	97149.52
JC + G	97177.86
JC + I	97686.81
GTR	101555.55
K2	102018.93
T92	102058.84
TN93	102112.41
HKY	102676.28
JC	102788.15
TN93 + G + I	141621.36
HKY + G	187524.18
TN93 + G	210027.56

[†] Bayesian Information Criterion scores, model with lowest score most adequately describes the substitution pattern.
[§] Gamma distribution.
[¶] Invariable rates among sites.

	G. graminis var. *avenae* CBS87.65	*G. graminis* var. *graminis* M33	*G. graminis* var. *graminis* AD1-2	*G.graminis* var. *graminis* M53	*G. graminis* var. *graminis* M54	*G. graminis* var. *graminis* GSGC15-3	*G. graminis* var. *graminis* GSGC15-4	*G. graminis* var. *graminis* CBS235.32	*G.graminis* var. *tritici* M55	*G.graminis* var. *tritici* r3111a	*G. paulograminis* DR10-3	*G. paulograminis* DR12-1	*G. paulograminis* DR13-1	*G. paulograminis* DR1-4	*G. paulograminis* DR6-4	*G. paulograminis* OW4-4	*G. paulograminis* RS7-1
G. graminis var. *avenae* CBS87.65																	
G. graminis var. *graminis* M33	0.045																
G. graminis var. *graminis* AD1-2	0.038	0.018															
G.graminis var. *graminis* M53	0.041	0.016	0.017														
G. graminis var. *graminis* M54	0.042	0.002	0.016	0.014													
G. graminis var. *graminis* GSGC15-3	0.038	0.018	0.000	0.017	0.016												
G. graminis var. *graminis* GSGC15-4	0.038	0.017	0.001	0.016	0.015	0.001											
G. graminis var. *graminis* CBS235.32	0.039	0.021	0.016	0.018	0.018	0.016	0.016										
G.graminis var. *tritici* M55	0.027	0.028	0.024	0.025	0.026	0.024	0.023	0.025									
G.graminis var. *tritici* r3111a	0.016	0.035	0.029	0.032	0.033	0.029	0.029	0.030	0.015								
G. paulograminis DR10-3	0.041	0.025	0.022	0.022	0.023	0.022	0.021	0.024	0.020	0.033							
G. paulograminis DR12-1	0.040	0.025	0.021	0.022	0.022	0.021	0.020	0.023	0.019	0.032	0.001						
G. paulograminis DR13-1	0.040	0.025	0.021	0.022	0.022	0.021	0.020	0.023	0.019	0.032	0.001	0.000					
G. paulograminis DR1-4	0.041	0.025	0.021	0.022	0.022	0.021	0.020	0.023	0.019	0.033	0.001	0.001	0.001				
G. paulograminis DR6-4	0.042	0.026	0.022	0.023	0.023	0.022	0.021	0.025	0.021	0.034	0.001	0.001	0.001	0.002			
G. paulograminis OW4-4	0.041	0.025	0.021	0.022	0.023	0.021	0.021	0.024	0.020	0.033	0.001	0.001	0.001	0.001	0.002		
G. paulograminis RS7-1	0.041	0.025	0.022	0.022	0.023	0.022	0.021	0.024	0.020	0.033	0.001	0.001	0.001	0.001	0.002	0.001	

Figure B.79 Estimates of evolutionary divergence between sequences for the combined ITS, LSU, SSU, *MCM7*, *TEF1*, and *RPB1* datasets of species of *Gaeumannomyces*.



	M. hawaiiensis KR10-6	*M. cynodontis* RS3-1	*M. cynodontis* RS5-5	*M. cynodontis* RS7-2	*M. incrustans* WW3-5	*M. incrustans* LC8-6	*M. incrustans* M35	*M. incrustans* M51	*M. incrustans* RRFMV14-5	*M. panicorum* CM2s8	*M. panicorum* CM7m9	*M. panicorum* CM9m11	*M. panicorum* CM10s2	*M. poae* M47	*M. poae* ATCC64411	*M. poae* TAP41	*M. poae* TAP35	*M. poae* SPKBG7	*M. rhizophila* M22	*M. rhizophila* M23	*M. taurocanis* GSGC10-2	*M. taurocanis* RRFCHMP1-3	*M. taurocanis* RRFMV10-2	*M. taurocanis* TPC5-3	*M. taurocanis* TPC4-5
M. hawaiiensis KR10-6																									
M. cynodontis RS3-1	0.012																								
M. cynodontis RS5-5	0.012	0.000																							
M. cynodontis RS7-2	0.012	0.000	0.000																						
M. incrustans WW3-5	0.031	0.027	0.027	0.027																					
M. incrustans LC8-6	0.033	0.027	0.027	0.027	0.005																				
M. incrustans M35	0.034	0.028	0.028	0.028	0.004	0.005																			
M. incrustans M51	0.035	0.029	0.029	0.029	0.004	0.007	0.005																		
M. incrustans RRFMV14-5	0.032	0.026	0.026	0.026	0.002	0.003	0.002	0.003																	
M. panicorum CM2s8	0.015	0.021	0.021	0.021	0.031	0.029	0.032	0.033	0.030																
M. panicorum CM7m9	0.015	0.021	0.021	0.021	0.031	0.028	0.031	0.032	0.029	0.003															
M. panicorum CM9m11	0.016	0.022	0.022	0.022	0.032	0.030	0.033	0.034	0.031	0.001	0.002														
M. panicorum CM10s2	0.015	0.021	0.021	0.021	0.031	0.028	0.031	0.032	0.029	0.003	0.000	0.002													
M. poae M47	0.027	0.033	0.033	0.033	0.026	0.023	0.025	0.026	0.024	0.023	0.022	0.024	0.022												
M. poae ATCC64411	0.027	0.033	0.033	0.033	0.026	0.023	0.025	0.026	0.024	0.023	0.022	0.024	0.022	0.000											
M. poae TAP41	0.033	0.027	0.027	0.027	0.020	0.018	0.020	0.020	0.019	0.030	0.029	0.031	0.029	0.008	0.008										
M. poae TAP35	0.033	0.026	0.026	0.026	0.019	0.017	0.019	0.019	0.018	0.029	0.028	0.030	0.028	0.007	0.007	0.002									
M. poae SPKBG7	0.026	0.033	0.033	0.033	0.026	0.024	0.026	0.026	0.025	0.023	0.021	0.023	0.021	0.002	0.002	0.008	0.008								
M. rhizophila M22	0.033	0.027	0.027	0.027	0.021	0.019	0.021	0.021	0.020	0.030	0.029	0.030	0.029	0.012	0.012	0.005	0.005	0.011							
M. rhizophila M23	0.033	0.026	0.026	0.026	0.020	0.018	0.021	0.020	0.019	0.029	0.029	0.030	0.029	0.011	0.011	0.005	0.004	0.010	0.001						
M. taurocanis GSGC10-2	0.009	0.005	0.005	0.005	0.027	0.026	0.027	0.028	0.025	0.020	0.021	0.022	0.021	0.032	0.032	0.026	0.025	0.032	0.026	0.026					
M. taurocanis RRFCHMP1-3	0.010	0.004	0.004	0.004	0.027	0.026	0.027	0.028	0.025	0.020	0.020	0.021	0.020	0.032	0.032	0.026	0.025	0.032	0.026	0.025	0.001				
M. taurocanis RRFMV10-2	0.009	0.005	0.005	0.005	0.027	0.026	0.027	0.028	0.025	0.020	0.021	0.022	0.021	0.032	0.032	0.026	0.025	0.032	0.026	0.026	0.000	0.001			
M. taurocanis TPC5-3	0.010	0.005	0.005	0.005	0.026	0.025	0.026	0.027	0.025	0.020	0.020	0.021	0.020	0.032	0.032	0.026	0.025	0.031	0.025	0.025	0.001	0.001	0.001		
M. taurocanis TPC4-5	0.010	0.005	0.005	0.005	0.026	0.025	0.026	0.027	0.025	0.020	0.020	0.021	0.020	0.032	0.032	0.026	0.025	0.031	0.025	0.025	0.001	0.001	0.001	0.000	

Figure B.80 Estimates of evolutionary divergence between sequences for the combined ITS, LSU, SSU, *MCM7*, *TEF1*, and *RPB1* datasets of species of *Magnaporthiopsis*.

	P. cynodontis RW3-4	*P. eragrostis* CM12m9	*P. eragrostis* CM20m5-2	*P. panicorum* CM3m7	*P. panicorum* CM9s6	*P. schizachyrii* AL3s4	*P. schizachyrii* AL2m1
P. cynodontis RW3-4							
P. eragrostis CM12m9	0.041						
P. eragrostis CM20m5-2	0.041	0.000					
P. panicorum CM3m7	0.041	0.019	0.019				
P. panicorum CM9s6	0.041	0.019	0.018	0.000			
P. schizachyrii AL3s4	0.042	0.011	0.011	0.020	0.020		
P. schizachyrii AL2m1	0.042	0.011	0.011	0.020	0.020	0.000	

Figure B.81 Estimates of evolutionary divergence between sequences for the combined ITS, LSU, SSU, *MCM7*, *TEF1*, and *RPB1* datasets of species of *Pseudophialophora*.

Table B.3 Maximum likelihood fits of nucleotide substitution models for the internal transcribed spacer (ITS) region dataset.

Model	BIC[†]
General Time Reversible (GTR) +G[§]	12668.46
Kimura 2-parameter (K2)+G	12668.84
GTR+G+I[¶]	12675.44
K2+G+I	12677.27
Tamura 3-parameter (T92)+G	12692.44
T92+G+I	12700.77
Tamura-Nei (TN93)+G	12729.10
TN93+G+I	12737.01
Hasegawa-Kishino-Yano (HKY)+G	12764.77
HKY+G+I	12773.36
Jukes-Cantor (JC)+G	12791.69
JC+G+I	12800.61
K2+I	12897.92
TN93+I	12921.28
GTR+I	12921.67
T92+I	12924.96
HKY+I	12947.86
JC+I	13008.74
TN93	13508.93
GTR	13514.01
K2	13520.37
T92	13552.04
HKY	13576.57
JC	13621.07

[†] Bayesian Information Criterion scores, model with lowest score most adequately describes the substitution pattern.
[§] Gamma distribution.
[¶] Invariable rates among sites.

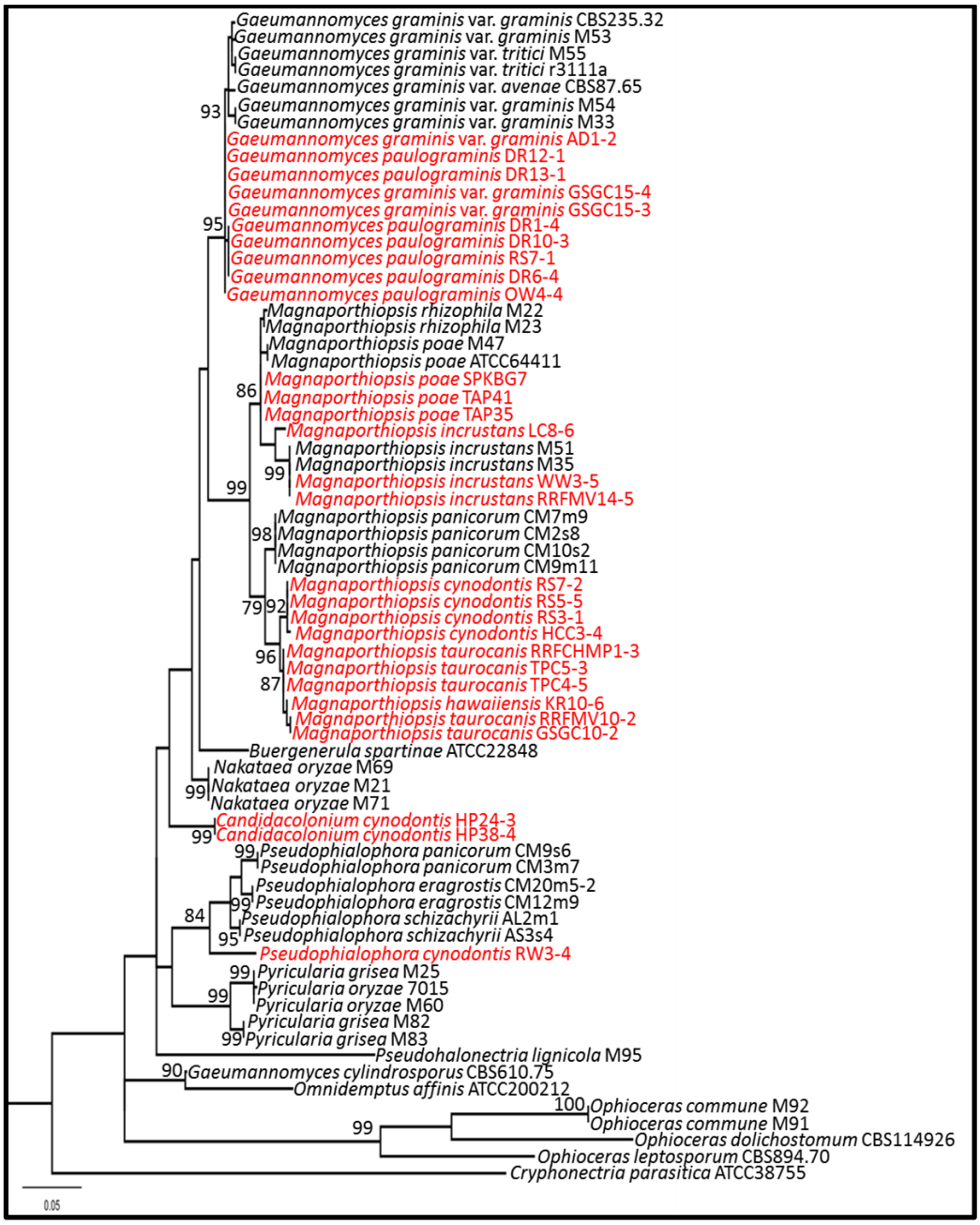
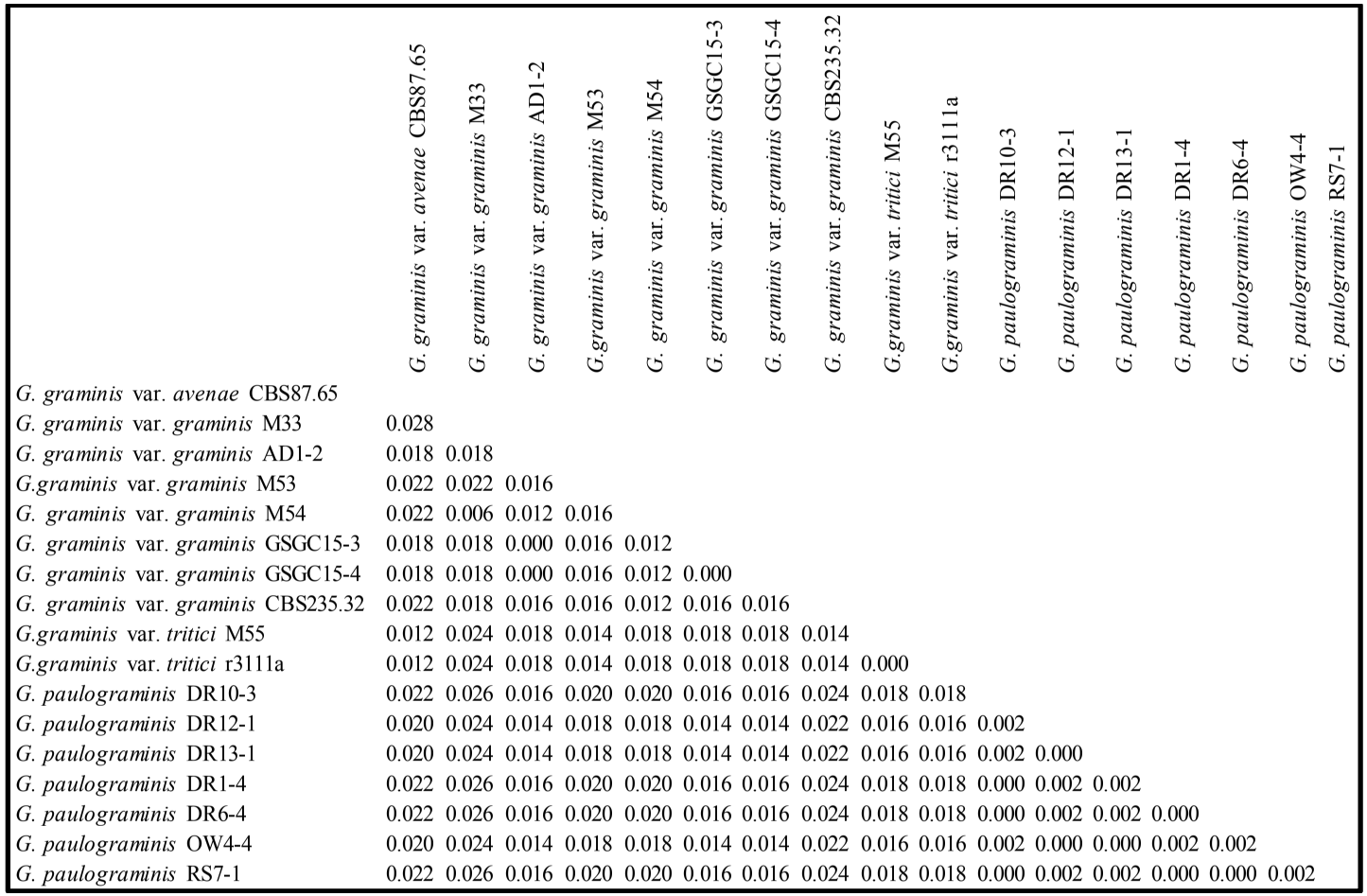


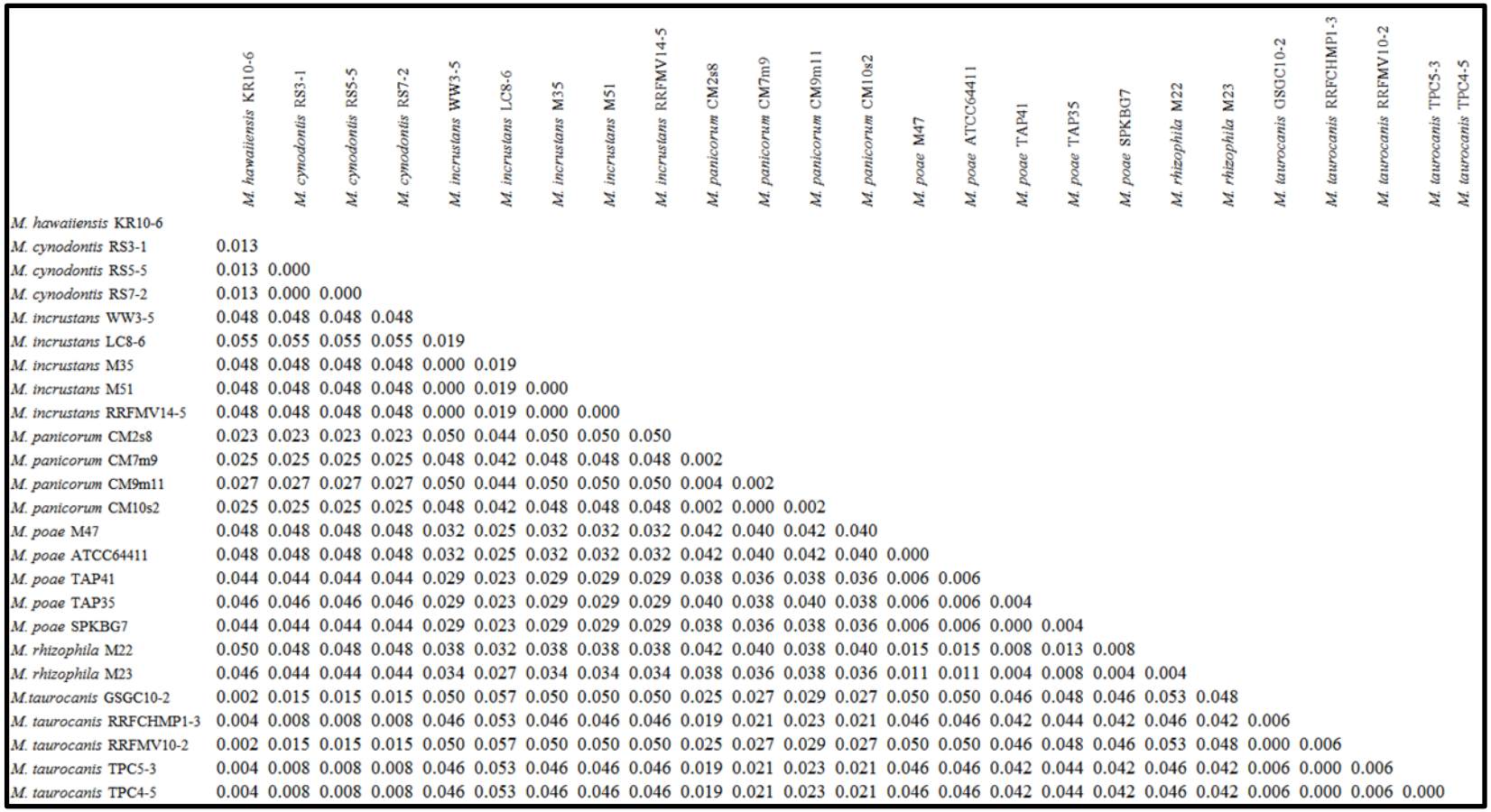
Figure B.82 Maximum Likelihood phylogram based on the internal transcribed spacer (ITS) region dataset.

ML bootstrap values ≥ 75% are presented above internodes. Sequences of isolates displayed in red were generated from this study. All other data were obtained from public databases. *Cryphonectria parasitica* was chosen as the outgroup taxon.



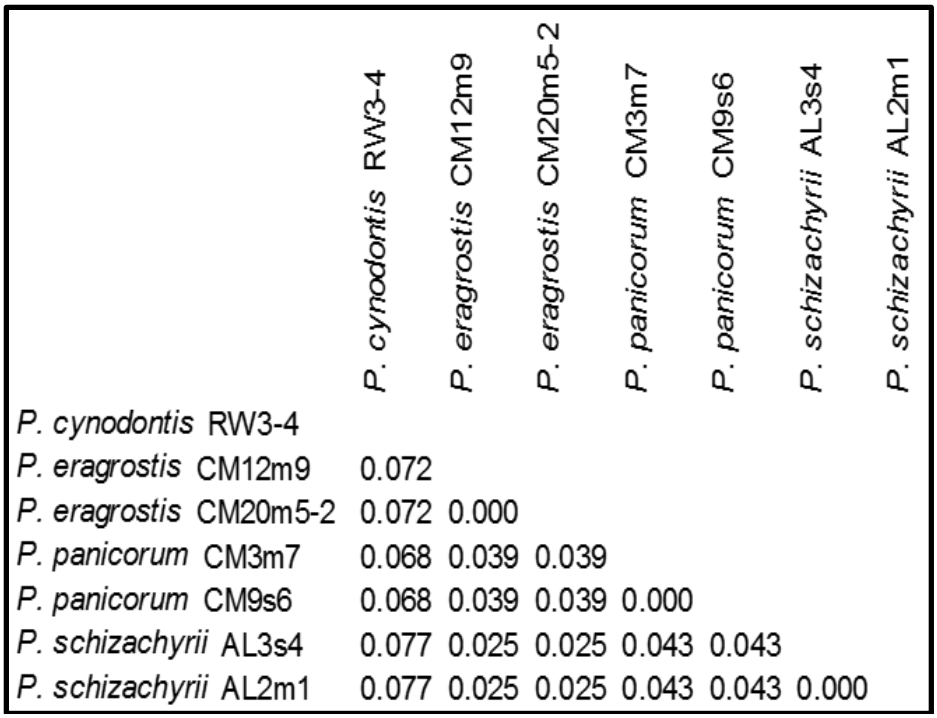
	G. graminis var. *avenae* CBS87.65	*G. graminis* var. *graminis* M33	*G. graminis* var. *graminis* AD1-2	*G.graminis* var. *graminis* M53	*G. graminis* var. *graminis* M54	*G. graminis* var. *graminis* GSGC15-3	*G. graminis* var. *graminis* GSGC15-4	*G. graminis* var. *graminis* CBS235.32	*G.graminis* var. *tritici* M55	*G.graminis* var. *tritici* r3111a	*G. paulograminis* DR10-3	*G. paulograminis* DR12-1	*G. paulograminis* DR13-1	*G. paulograminis* DR1-4	*G. paulograminis* DR6-4	*G. paulograminis* OW4-4	*G. paulograminis* RS7-1
G. graminis var. *avenae* CBS87.65																	
G. graminis var. *graminis* M33	0.028																
G. graminis var. *graminis* AD1-2	0.018	0.018															
G.graminis var. *graminis* M53	0.022	0.022	0.016														
G. graminis var. *graminis* M54	0.022	0.006	0.012	0.016													
G. graminis var. *graminis* GSGC15-3	0.018	0.018	0.000	0.016	0.012												
G. graminis var. *graminis* GSGC15-4	0.018	0.018	0.000	0.016	0.012	0.000											
G. graminis var. *graminis* CBS235.32	0.022	0.018	0.016	0.016	0.012	0.016	0.016										
G.graminis var. *tritici* M55	0.012	0.024	0.018	0.014	0.018	0.018	0.018	0.014									
G.graminis var. *tritici* r3111a	0.012	0.024	0.018	0.014	0.018	0.018	0.018	0.014	0.000								
G. paulograminis DR10-3	0.022	0.026	0.016	0.020	0.020	0.016	0.016	0.024	0.018	0.018							
G. paulograminis DR12-1	0.020	0.024	0.014	0.018	0.018	0.014	0.014	0.022	0.016	0.016	0.002						
G. paulograminis DR13-1	0.020	0.024	0.014	0.018	0.018	0.014	0.014	0.022	0.016	0.016	0.002	0.000					
G. paulograminis DR1-4	0.022	0.026	0.016	0.020	0.020	0.016	0.016	0.024	0.018	0.018	0.000	0.002	0.002				
G. paulograminis DR6-4	0.022	0.026	0.016	0.020	0.020	0.016	0.016	0.024	0.018	0.018	0.000	0.002	0.002	0.000			
G. paulograminis OW4-4	0.020	0.024	0.014	0.018	0.018	0.014	0.014	0.022	0.016	0.016	0.002	0.000	0.000	0.002	0.002		
G. paulograminis RS7-1	0.022	0.026	0.016	0.020	0.020	0.016	0.016	0.024	0.018	0.018	0.000	0.002	0.002	0.000	0.000	0.002	

Figure B.83 Estimates of evolutionary divergence between sequences for the internal transcribed spacer (ITS) region of species of *Gaeumannomyces*.



	M. hawaiiensis KR10-6	*M. cynodontis* RS3-1	*M. cynodontis* RS5-5	*M. cynodontis* RS7-2	*M. incrustans* WW3-5	*M. incrustans* LC8-6	*M. incrustans* M35	*M. incrustans* M51	*M. incrustans* RRFMV14-5	*M. panicorum* CM2s8	*M. panicorum* CM7m9	*M. panicorum* CM9m11	*M. panicorum* CM10s2	*M. poae* M47	*M. poae* ATCC64411	*M. poae* TAP41	*M. poae* TAP35	*M. poae* SPKBG7	*M. rhizophila* M22	*M. rhizophila* M23	*M. taurocanis* GSGC10-2	*M. taurocanis* RRFCHMP1-3	*M. taurocanis* RRFMV10-2	*M. taurocanis* TPC5-3	*M. taurocanis* TPC4-5
M. hawaiiensis KR10-6																									
M. cynodontis RS3-1	0.013																								
M. cynodontis RS5-5	0.013	0.000																							
M. cynodontis RS7-2	0.013	0.000	0.000																						
M. incrustans WW3-5	0.048	0.048	0.048	0.048																					
M. incrustans LC8-6	0.055	0.055	0.055	0.055	0.019																				
M. incrustans M35	0.048	0.048	0.048	0.048	0.000	0.019																			
M. incrustans M51	0.048	0.048	0.048	0.048	0.000	0.019	0.000																		
M. incrustans RRFMV14-5	0.048	0.048	0.048	0.048	0.000	0.019	0.000	0.000																	
M. panicorum CM2s8	0.023	0.023	0.023	0.023	0.050	0.044	0.050	0.050	0.050																
M. panicorum CM7m9	0.025	0.025	0.025	0.025	0.048	0.042	0.048	0.048	0.048	0.002															
M. panicorum CM9m11	0.027	0.027	0.027	0.027	0.050	0.044	0.050	0.050	0.050	0.004	0.002														
M. panicorum CM10s2	0.025	0.025	0.025	0.025	0.048	0.042	0.048	0.048	0.048	0.002	0.000	0.002													
M. poae M47	0.048	0.048	0.048	0.048	0.032	0.025	0.032	0.032	0.032	0.042	0.040	0.042	0.040												
M. poae ATCC64411	0.048	0.048	0.048	0.048	0.032	0.025	0.032	0.032	0.032	0.042	0.040	0.042	0.040	0.000											
M. poae TAP41	0.044	0.044	0.044	0.044	0.029	0.023	0.029	0.029	0.029	0.038	0.036	0.038	0.036	0.006	0.006										
M. poae TAP35	0.046	0.046	0.046	0.046	0.029	0.023	0.029	0.029	0.029	0.040	0.038	0.040	0.038	0.006	0.006	0.004									
M. poae SPKBG7	0.044	0.044	0.044	0.044	0.029	0.023	0.029	0.029	0.029	0.038	0.036	0.038	0.036	0.006	0.006	0.000	0.004								
M. rhizophila M22	0.050	0.048	0.048	0.048	0.038	0.032	0.038	0.038	0.038	0.042	0.040	0.038	0.040	0.015	0.015	0.008	0.013	0.008							
M. rhizophila M23	0.046	0.044	0.044	0.044	0.034	0.027	0.034	0.034	0.034	0.038	0.036	0.038	0.036	0.011	0.011	0.004	0.008	0.004	0.004						
M.taurocanis GSGC10-2	0.002	0.015	0.015	0.015	0.050	0.057	0.050	0.050	0.050	0.025	0.027	0.029	0.027	0.050	0.050	0.046	0.048	0.046	0.053	0.048					
M. taurocanis RRFCHMP1-3	0.004	0.008	0.008	0.008	0.046	0.053	0.046	0.046	0.046	0.019	0.021	0.023	0.021	0.046	0.046	0.042	0.044	0.042	0.046	0.042	0.006				
M. taurocanis RRFMV10-2	0.002	0.015	0.015	0.015	0.050	0.057	0.050	0.050	0.050	0.025	0.027	0.029	0.027	0.050	0.050	0.046	0.048	0.046	0.053	0.048	0.000	0.006			
M. taurocanis TPC5-3	0.004	0.008	0.008	0.008	0.046	0.053	0.046	0.046	0.046	0.019	0.021	0.023	0.021	0.046	0.046	0.042	0.044	0.042	0.046	0.042	0.006	0.000	0.006		
M. taurocanis TPC4-5	0.004	0.008	0.008	0.008	0.046	0.053	0.046	0.046	0.046	0.019	0.021	0.023	0.021	0.046	0.046	0.042	0.044	0.042	0.046	0.042	0.006	0.000	0.006	0.000	

Figure B.84 Estimates of evolutionary divergence between sequences for the internal transcribed spacer (ITS) region of species of *Magnaporthiopsis*.



	P. cynodontis RW3-4	*P. eragrostis* CM12m9	*P. eragrostis* CM20m5-2	*P. panicorum* CM3m7	*P. panicorum* CM9s6	*P. schizachyrii* AL3s4	*P. schizachyrii* AL2m1
P. cynodontis RW3-4							
P. eragrostis CM12m9	0.072						
P. eragrostis CM20m5-2	0.072	0.000					
P. panicorum CM3m7	0.068	0.039	0.039				
P. panicorum CM9s6	0.068	0.039	0.039	0.000			
P. schizachyrii AL3s4	0.077	0.025	0.025	0.043	0.043		
P. schizachyrii AL2m1	0.077	0.025	0.025	0.043	0.043	0.000	

Figure B.85 Estimates of evolutionary divergence between sequences for the internal transcribed spacer (ITS) region of species of *Pseudophialophora*.

Table B.4 Maximum likelihood fits of nucleotide substitution models for the large subunit (LSU) gene dataset.

Model	BIC[†]
Kimura 2-parameter (K2)+G[§]+I[¶]	8885.70
General Time Reversible (GTR)+G+I	8887.82
Tamura 3-parameter (T92)+G+I	8889.62
GTR+G	8892.28
K2+G	8898.85
T92+G	8902.71
Tamura-Nei (TN93)+I	8937.07
K2+I	8942.73
T92+I	8945.99
GTR+I	8957.23
Hasegawa-Kishino-Yano (HKY)+I	8996.30
Jukes-Cantor (JC)+G+I	9034.24
JC+G	9045.33
JC+I	9087.01
TN93	9300.63
GTR	9319.03
K2	9357.40
T92	9365.14
HKY	9427.72
JC	9493.92
TN93+G+I	31602.74
TN93+G	31607.26
HKY+G+I	31667.51
HKY+G	31681.73

[†] Bayesian Information Criterion scores, model with lowest score most adequately describes the substitution pattern.
[§] Gamma distribution.
[¶] Invariable rates among sites.

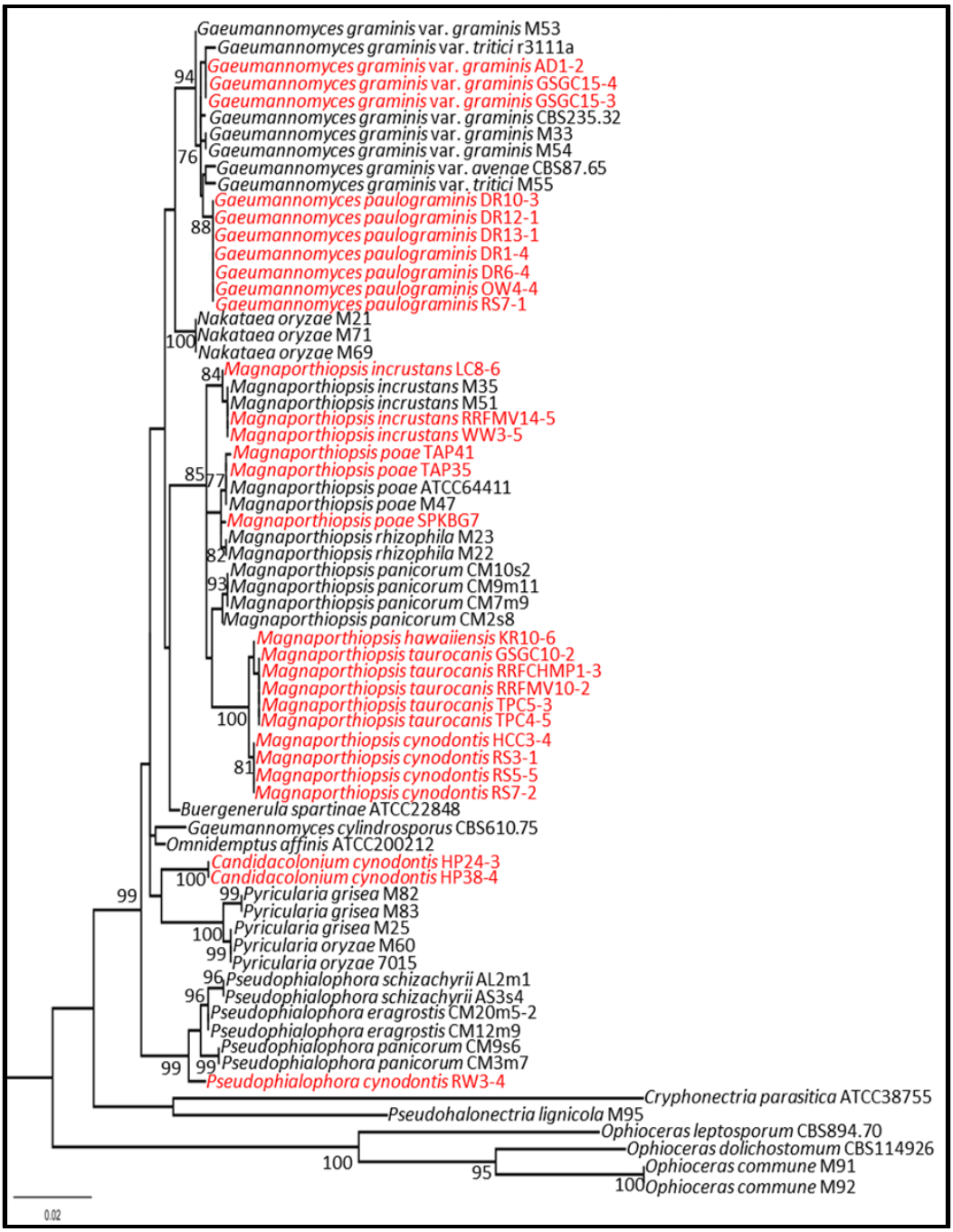
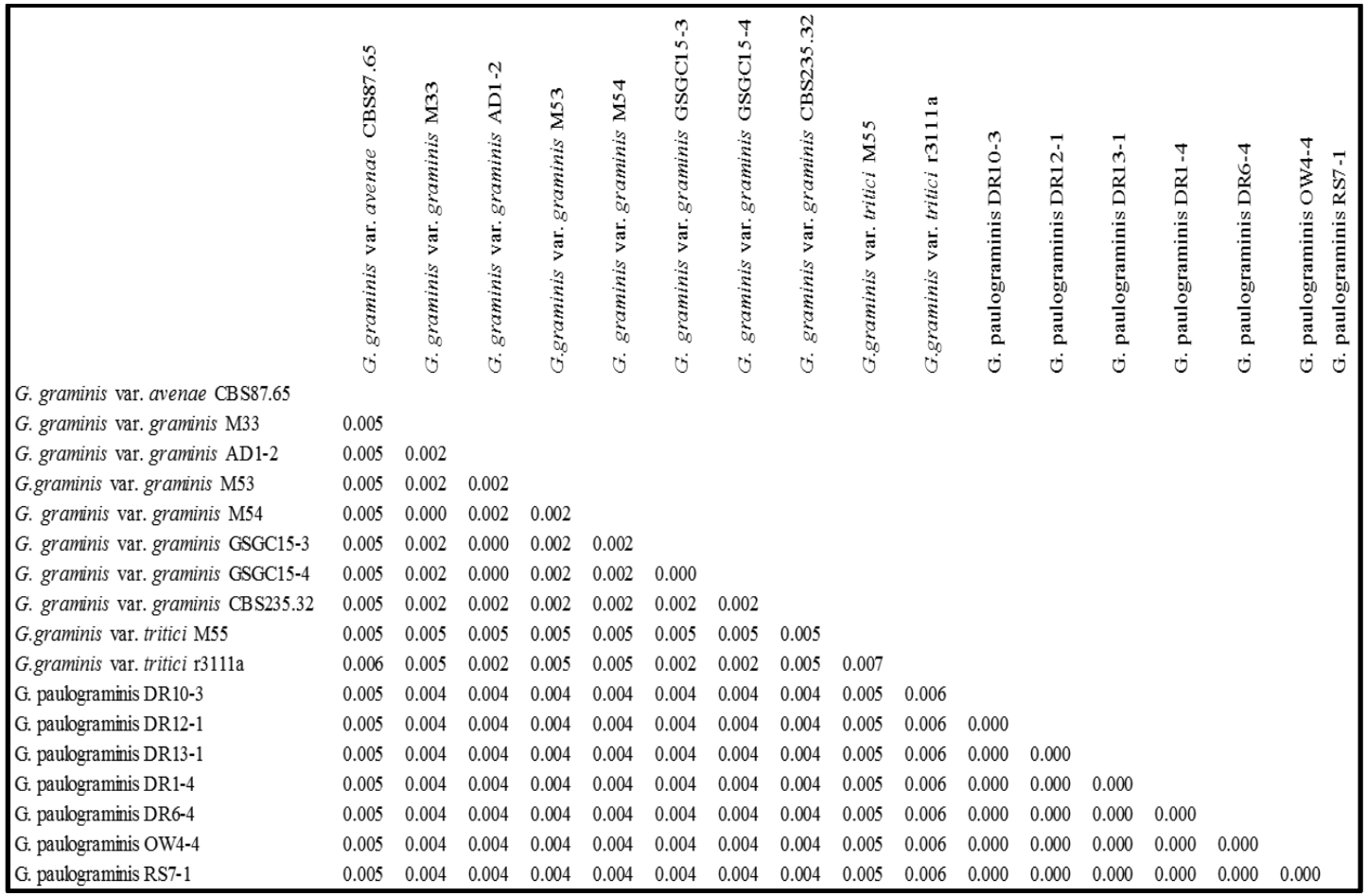


Figure B.86 Maximum Likelihood phylogram based on the large subunit (LSU) gene dataset.

ML bootstrap values ≥ 75% are presented above internodes. Sequences of isolates displayed in red were generated from this study. All other data were obtained from public databases. *Cryphonectria parasitica* was chosen as the outgroup taxon.



	G. graminis var. *avenae* CBS87.65	*G. graminis* var. *graminis* M33	*G. graminis* var. *graminis* AD1-2	*G.graminis* var. *graminis* M53	*G. graminis* var. *graminis* M54	*G. graminis* var. *graminis* GSGC15-3	*G. graminis* var. *graminis* GSGC15-4	*G. graminis* var. *graminis* CBS235.32	*G.graminis* var. *tritici* M55	*G.graminis* var. *tritici* r3111a	G. paulograminis DR10-3	G. paulograminis DR12-1	G. paulograminis DR13-1	G. paulograminis DR1-4	G. paulograminis DR6-4	G. paulograminis OW4-4	G. paulograminis RS7-1
G. graminis var. *avenae* CBS87.65																	
G. graminis var. *graminis* M33	0.005																
G. graminis var. *graminis* AD1-2	0.005	0.002															
G.graminis var. *graminis* M53	0.005	0.002	0.002														
G. graminis var. *graminis* M54	0.005	0.000	0.002	0.002													
G. graminis var. *graminis* GSGC15-3	0.005	0.002	0.000	0.002	0.002												
G. graminis var. *graminis* GSGC15-4	0.005	0.002	0.000	0.002	0.002	0.000											
G. graminis var. *graminis* CBS235.32	0.005	0.002	0.002	0.002	0.002	0.002	0.002										
G.graminis var. *tritici* M55	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005									
G.graminis var. *tritici* r3111a	0.006	0.005	0.002	0.005	0.005	0.002	0.002	0.005	0.007								
G. paulograminis DR10-3	0.005	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.005	0.006							
G. paulograminis DR12-1	0.005	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.005	0.006	0.000						
G. paulograminis DR13-1	0.005	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.005	0.006	0.000	0.000					
G. paulograminis DR1-4	0.005	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.005	0.006	0.000	0.000	0.000				
G. paulograminis DR6-4	0.005	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.005	0.006	0.000	0.000	0.000	0.000			
G. paulograminis OW4-4	0.005	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.005	0.006	0.000	0.000	0.000	0.000	0.000		
G. paulograminis RS7-1	0.005	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.005	0.006	0.000	0.000	0.000	0.000	0.000	0.000	

Figure B.87 Estimates of evolutionary divergence between sequences for the large subunit (LSU) gene of species of *Gaeumannomyces*.

	M. hawaiiensis KR10-6	*M. cynodontis* RS3-1	*M. cynodontis* RS5-5	*M. cynodontis* RS7-2	*M. incrustans* WW3-5	*M. incrustans* LC8-6	*M. incrustans* M35	*M. incrustans* M51	*M. incrustans* RRFMV14-5	*M. panicorum* CM2s8	*M. panicorum* CM7m9	*M. panicorum* CM9m11	*M. panicorum* CM10s2	*M. poae* M47	*M. poae* ATCC64411	*M. poae* TAP41	*M. poae* TAP35	*M. poae* SPKBG7	*M. rhizophila* M22	*M. rhizophila* M23	*M. taurocanis* GSGC10-2	*M. taurocanis* RRFCHMP1-3	*M. taurocanis* RRFMV10-2	*M. taurocanis* TPC5-3	*M. taurocanis* TPC4-5
M. hawaiiensis KR10-6																									
M. cynodontis RS3-1	0.002																								
M. cynodontis RS5-5	0.002	0.000																							
M. cynodontis RS7-2	0.002	0.000	0.000																						
M. incrustans WW3-5	0.018	0.018	0.018	0.018																					
M. incrustans LC8-6	0.017	0.017	0.017	0.017	0.001																				
M. incrustans M35	0.018	0.018	0.018	0.018	0.000	0.001																			
M. incrustans M51	0.018	0.018	0.018	0.018	0.000	0.001	0.000																		
M. incrustans RRFMV14-5	0.018	0.018	0.018	0.018	0.000	0.001	0.000	0.000																	
M. panicorum CM2s8	0.012	0.012	0.012	0.012	0.011	0.009	0.011	0.011	0.011																
M. panicorum CM7m9	0.013	0.013	0.013	0.013	0.012	0.011	0.012	0.012	0.012	0.001															
M. panicorum CM9m11	0.013	0.013	0.013	0.013	0.012	0.011	0.012	0.012	0.012	0.001	0.000														
M. panicorum CM10s2	0.013	0.013	0.013	0.013	0.012	0.011	0.012	0.012	0.012	0.001	0.000	0.000													
M. poae M47	0.015	0.015	0.015	0.015	0.009	0.008	0.009	0.009	0.009	0.008	0.009	0.009	0.009												
M. poae ATCC64411	0.015	0.015	0.015	0.015	0.009	0.008	0.009	0.009	0.009	0.008	0.009	0.009	0.009	0.000											
M. poae TAP41	0.017	0.017	0.017	0.017	0.011	0.009	0.011	0.011	0.011	0.009	0.011	0.011	0.011	0.001	0.001										
M. poae TAP35	0.015	0.015	0.015	0.015	0.009	0.008	0.009	0.009	0.009	0.008	0.009	0.009	0.009	0.000	0.000	0.001									
M. poae SPKBG7	0.015	0.015	0.015	0.015	0.012	0.011	0.012	0.012	0.012	0.008	0.007	0.007	0.007	0.004	0.004	0.005	0.004								
M. rhizophila M22	0.017	0.017	0.017	0.017	0.011	0.009	0.011	0.011	0.011	0.009	0.011	0.011	0.011	0.002	0.002	0.004	0.002	0.004							
M. rhizophila M23	0.017	0.017	0.017	0.017	0.011	0.009	0.011	0.011	0.011	0.009	0.011	0.011	0.011	0.002	0.002	0.004	0.002	0.004	0.000						
M. taurocanis GSGC10-2	0.001	0.004	0.004	0.004	0.019	0.018	0.019	0.019	0.019	0.013	0.014	0.014	0.014	0.017	0.017	0.018	0.017	0.017	0.018	0.018					
M. taurocanis RRFCHMP1-3	0.001	0.004	0.004	0.004	0.019	0.018	0.019	0.019	0.019	0.013	0.014	0.014	0.014	0.017	0.017	0.018	0.017	0.017	0.018	0.018	0.000				
M. taurocanis RRFMV10-2	0.001	0.004	0.004	0.004	0.019	0.018	0.019	0.019	0.019	0.013	0.014	0.014	0.014	0.017	0.017	0.018	0.017	0.017	0.018	0.018	0.000	0.000			
M. taurocanis TPC5-3	0.001	0.004	0.004	0.004	0.019	0.018	0.019	0.019	0.019	0.013	0.014	0.014	0.014	0.017	0.017	0.018	0.017	0.017	0.018	0.018	0.000	0.000	0.000		
M. taurocanis TPC4-5	0.001	0.004	0.004	0.004	0.019	0.018	0.019	0.019	0.019	0.013	0.014	0.014	0.014	0.017	0.017	0.018	0.017	0.017	0.018	0.018	0.000	0.000	0.000	0.000	

Figure B.88 Estimates of evolutionary divergence between sequences for the large subunit (LSU) gene of species of *Magnaporthiopsis*.

	P. cynodontis RW3-4	*P. eragrostis* CM12m9	*P. eragrostis* CM20m5-2	*P. panicorum* CM3m7	*P. panicorum* CM9s6	*P. schizachyrii* AL3s4	*P. schizachyrii* AL2m1
P. cynodontis RW3-4							
P. eragrostis CM12m9	0.008						
P. eragrostis CM20m5-2	0.008	0.000					
P. panicorum CM3m7	0.011	0.007	0.007				
P. panicorum CM9s6	0.011	0.007	0.007	0.000			
P. schizachyrii AL3s4	0.012	0.004	0.004	0.011	0.011		
P. schizachyrii AL2m1	0.012	0.004	0.004	0.011	0.011	0.000	

Figure B.89 Estimates of evolutionary divergence between sequences for the large subunit (LSU) gene of species of *Pseudophialophora*.

Table B.5 Maximum likelihood fits of nucleotide substitution models for the small subunit (SSU) gene dataset.

Model	BIC[†]
Kimura 2-parameter (K2) + G	4096.06
Tamura 3-parameter (T92) + G	4103.97
K2+G+I	4105.26
T92 + I	4107.17
K2+I	4109.20
T92+G+I	4113.33
K2	4115.70
T92	4123.70
Hasegawa-Kishino-Yano (HKY)+G	4125.17
HKY+I	4126.47
Tamura-Nei (TN93) + G	4131.58
TN93+I	4132.90
Jukes-Cantor (JC)+G	4133.57
HKY+G+I	4134.43
TN93+G+I	4140.77
JC+G+I	4143.06
HKY	4145.08
TN93	4150.14
JC	4151.42
JC+I	4152.06
General Time Reversible (GTR)+G	4157.36
GTR+G+I	4166.66
GTR+I	4175.65
GTR	4180.16

[†] Bayesian Information Criterion scores, model with lowest score most adequately describes the substitution pattern.
[§] Gamma distribution.
[¶] Invariable rates among sites.

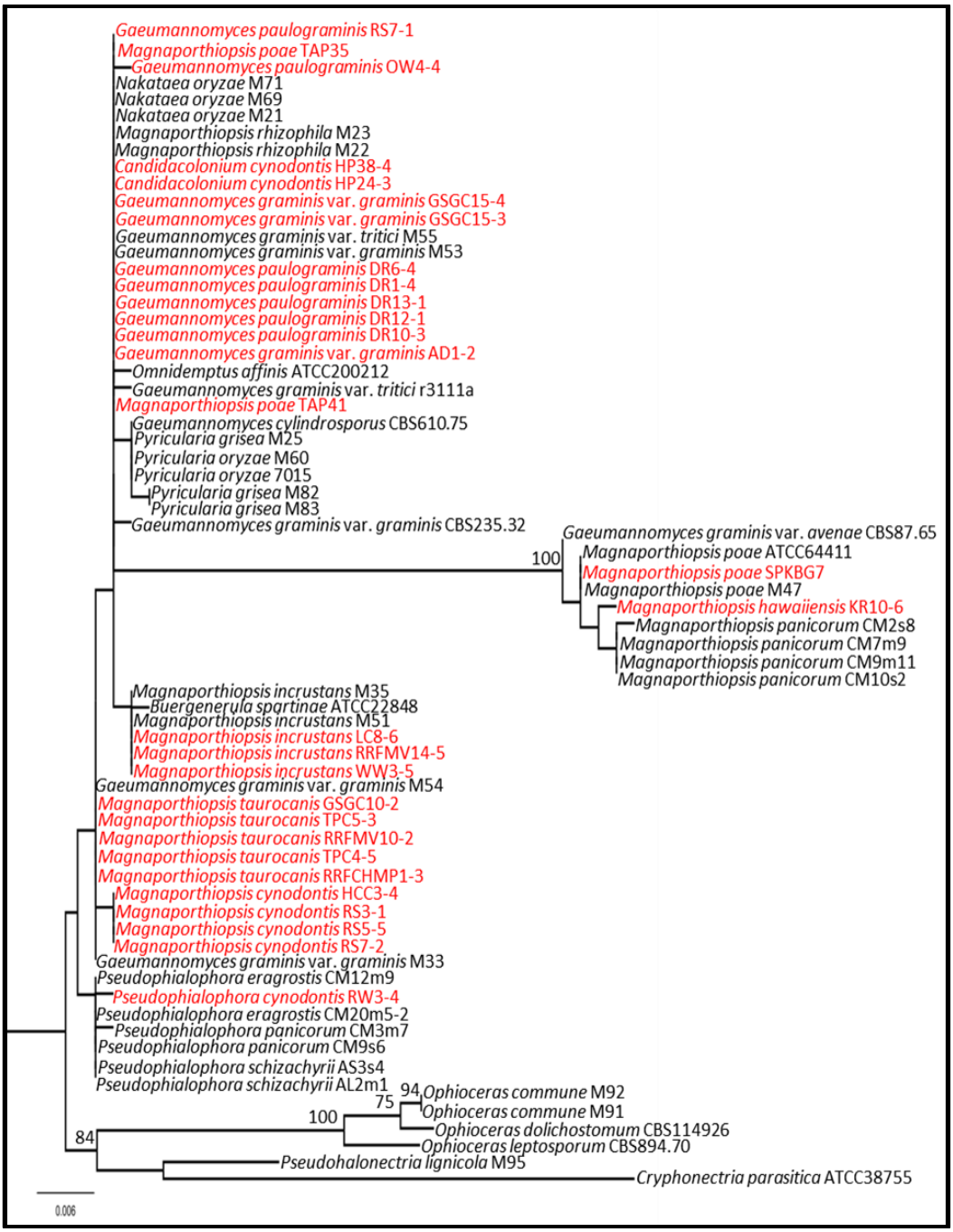
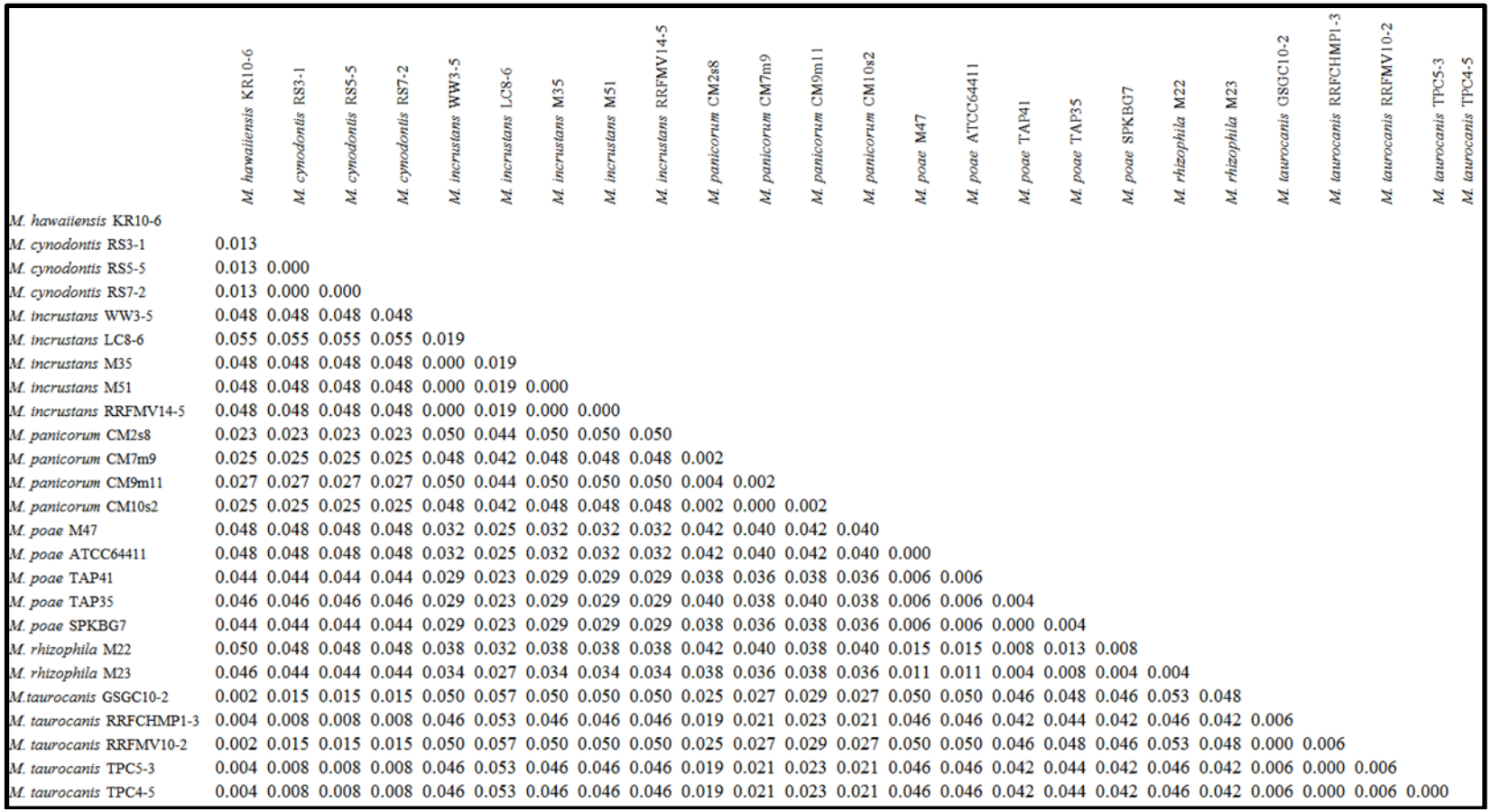


Figure B.90 Maximum Likelihood phylogram based on the small subunit (SSU) gene dataset.

ML bootstrap values ≥ 75% are presented above internodes. Sequences of isolates displayed in red were generated from this study. All other data were obtained from public databases. *Cryphonectria parasitica* was chosen as the outgroup taxon.

	G. graminis var. *avenae* CBS87.65	*G. graminis* var. *graminis* M33	*G. graminis* var. *graminis* AD1-2	*G.graminis* var. *graminis* M53	*G. graminis* var. *graminis* M54	*G. graminis* var. *graminis* GSGC15-3	*G. graminis* var. *graminis* GSGC15-4	*G. graminis* var. *graminis* CBS235.32	*G.graminis* var. *tritici* M55	*G.graminis* var. *tritici* r3111a	*G. paulograminis* DR10-3	*G. paulograminis* DR12-1	*G. paulograminis* DR13-1	*G. paulograminis* DR1-4	*G. paulograminis* DR6-4	*G. paulograminis* OW4-4	*G. paulograminis* RS7-1
G. graminis var. *avenae* CBS87.65																	
G. graminis var. *graminis* M33	0.048																
G. graminis var. *graminis* AD1-2	0.046	0.002															
G.graminis var. *graminis* M53	0.048	0.000	0.002														
G. graminis var. *graminis* M54	0.046	0.002	0.000	0.002													
G. graminis var. *graminis* GSGC15-3	0.046	0.002	0.000	0.002	0.000												
G. graminis var. *graminis* GSGC15-4	0.046	0.002	0.000	0.002	0.000	0.000											
G. graminis var. *graminis* CBS235.32	0.048	0.004	0.002	0.004	0.002	0.002	0.002										
G.graminis var. *tritici* M55	0.046	0.002	0.000	0.002	0.000	0.000	0.000	0.002									
G.graminis var. *tritici* r3111a	0.048	0.004	0.002	0.004	0.002	0.002	0.002	0.004	0.002								
G. paulograminis DR10-3	0.046	0.002	0.000	0.002	0.000	0.000	0.000	0.002	0.000	0.002							
G. paulograminis DR12-1	0.046	0.002	0.000	0.002	0.000	0.000	0.000	0.002	0.000	0.002	0.000						
G. paulograminis DR13-1	0.046	0.002	0.000	0.002	0.000	0.000	0.000	0.002	0.000	0.002	0.000	0.000					
G. paulograminis DR1-4	0.046	0.002	0.000	0.002	0.000	0.000	0.000	0.002	0.000	0.002	0.000	0.000	0.000				
G. paulograminis DR6-4	0.046	0.002	0.000	0.002	0.000	0.000	0.000	0.002	0.000	0.002	0.000	0.000	0.000	0.000			
G. paulograminis OW4-4	0.048	0.004	0.002	0.004	0.002	0.002	0.002	0.004	0.002	0.004	0.002	0.002	0.002	0.002	0.002		
G. paulograminis RS7-1	0.046	0.002	0.000	0.002	0.000	0.000	0.000	0.002	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.002	

Figure B.91 Estimates of evolutionary divergence between sequences for the small subunit (SSU) gene of species of *Gaeumannomyces*.



	M. hawaiiensis KR10-6	*M. cynodontis* RS3-1	*M. cynodontis* RS5-5	*M. cynodontis* RS7-2	*M. incrustans* WW3-5	*M. incrustans* LC8-6	*M. incrustans* M35	*M. incrustans* M51	*M. incrustans* RRFMV14-5	*M. panicorum* CM2s8	*M. panicorum* CM7m9	*M. panicorum* CM9m11	*M. panicorum* CM10s2	*M. poae* M47	*M. poae* ATCC64411	*M. poae* TAP41	*M. poae* TAP35	*M. poae* SPKBG7	*M. rhizophila* M22	*M. rhizophila* M23	*M. taurocanis* GSGC10-2	*M. taurocanis* RRFCHMP1-3	*M. taurocanis* RRFMV10-2	*M. taurocanis* TPC5-3	*M. taurocanis* TPC4-5
M. hawaiiensis KR10-6																									
M. cynodontis RS3-1	0.013																								
M. cynodontis RS5-5	0.013	0.000																							
M. cynodontis RS7-2	0.013	0.000	0.000																						
M. incrustans WW3-5	0.048	0.048	0.048	0.048																					
M. incrustans LC8-6	0.055	0.055	0.055	0.055	0.019																				
M. incrustans M35	0.048	0.048	0.048	0.048	0.000	0.019																			
M. incrustans M51	0.048	0.048	0.048	0.048	0.000	0.019	0.000																		
M. incrustans RRFMV14-5	0.048	0.048	0.048	0.048	0.000	0.019	0.000	0.000																	
M. panicorum CM2s8	0.023	0.023	0.023	0.023	0.050	0.044	0.050	0.050	0.050																
M. panicorum CM7m9	0.025	0.025	0.025	0.025	0.048	0.042	0.048	0.048	0.048	0.002															
M. panicorum CM9m11	0.027	0.027	0.027	0.027	0.050	0.044	0.050	0.050	0.050	0.004	0.002														
M. panicorum CM10s2	0.025	0.025	0.025	0.025	0.048	0.042	0.048	0.048	0.048	0.002	0.000	0.002													
M. poae M47	0.048	0.048	0.048	0.048	0.032	0.025	0.032	0.032	0.032	0.042	0.040	0.042	0.040												
M. poae ATCC64411	0.048	0.048	0.048	0.048	0.032	0.025	0.032	0.032	0.032	0.042	0.040	0.042	0.040	0.000											
M. poae TAP41	0.044	0.044	0.044	0.044	0.029	0.023	0.029	0.029	0.029	0.038	0.036	0.038	0.036	0.006	0.006										
M. poae TAP35	0.046	0.046	0.046	0.046	0.029	0.023	0.029	0.029	0.029	0.040	0.038	0.040	0.038	0.006	0.006	0.004									
M. poae SPKBG7	0.044	0.044	0.044	0.044	0.029	0.023	0.029	0.029	0.029	0.038	0.036	0.038	0.036	0.006	0.006	0.000	0.004								
M. rhizophila M22	0.050	0.048	0.048	0.048	0.038	0.032	0.038	0.038	0.038	0.042	0.040	0.038	0.040	0.015	0.015	0.008	0.013	0.008							
M. rhizophila M23	0.046	0.044	0.044	0.044	0.034	0.027	0.034	0.034	0.034	0.038	0.036	0.038	0.036	0.011	0.011	0.004	0.008	0.004	0.004						
M.taurocanis GSGC10-2	0.002	0.015	0.015	0.015	0.050	0.057	0.050	0.050	0.050	0.025	0.027	0.029	0.027	0.050	0.050	0.046	0.048	0.046	0.053	0.048					
M. taurocanis RRFCHMP1-3	0.004	0.008	0.008	0.008	0.046	0.053	0.046	0.046	0.046	0.019	0.021	0.023	0.021	0.046	0.046	0.042	0.044	0.042	0.046	0.042	0.006				
M. taurocanis RRFMV10-2	0.002	0.015	0.015	0.015	0.050	0.057	0.050	0.050	0.050	0.025	0.027	0.029	0.027	0.050	0.050	0.046	0.048	0.046	0.053	0.048	0.000	0.006			
M. taurocanis TPC5-3	0.004	0.008	0.008	0.008	0.046	0.053	0.046	0.046	0.046	0.019	0.021	0.023	0.021	0.046	0.046	0.042	0.044	0.042	0.046	0.042	0.006	0.000	0.006		
M. taurocanis TPC4-5	0.004	0.008	0.008	0.008	0.046	0.053	0.046	0.046	0.046	0.019	0.021	0.023	0.021	0.046	0.046	0.042	0.044	0.042	0.046	0.042	0.006	0.000	0.006	0.000	

Figure B.92 Estimates of evolutionary divergence between sequences for the small subunit (SSU) gene of species of *Magnaporthiopsis*.

	P. cynodontis RW3-4	*P. eragrostis* CM12m9	*P. eragrostis* CM20m5-2	*P. panicorum* CM3m7	*P. panicorum* CM9s6	*P. schizachyrii* AL3s4	*P. schizachyrii* AL2m1
P. cynodontis RW3-4							
P. eragrostis CM12m9	0.002						
P. eragrostis CM20m5-2	0.002	0.000					
P. panicorum CM3m7	0.004	0.002	0.002				
P. panicorum CM9s6	0.002	0.000	0.000	0.002			
P. schizachyrii AL3s4	0.002	0.000	0.000	0.002	0.000		
P. schizachyrii AL2m1	0.002	0.000	0.000	0.002	0.000	0.000	

Figure B.93 Estimates of evolutionary divergence between sequences for the small subunit (SSU) gene of species of *Pseudophialophora*.

Table B.6 Maximum likelihood fits of nucleotide substitution models for the minichromosome maintenance complex component 7 (*MCM7*) gene dataset.

Model	BIC[†]
Tamura 3-parameter (T92) + G	9105.55
T92+G+I	9113.03
Hasegawa-Kishino-Yano (HKY)+G	9119.46
Tamura-Nei (TN93)+G	9127.27
HKY+G+I	9127.45
TN93+G+I	9134.21
Kimura 2-parameter (K2) + G	9146.06
K2+G+I	9154.32
General Time Reversible (GTR) + G	9154.50
GTR+G+I	9161.75
T92+I	9274.89
TN93+I	9288.57
HKY+I	9291.18
K2+I	9295.61
GTR+I	9310.33
Jukes-Cantor (JC)+G	9310.91
JC+G+I	9320.07
JC+I	9450.35
K2	9764.55
T92	9798.91
GTR	9802.81
TN93	9813.32
HKY	9820.29
JC	9913.63

[†] Bayesian Information Criterion scores, model with lowest score most adequately describes the substitution pattern.
[§] Gamma distribution.
[¶] Invariable rates among sites.

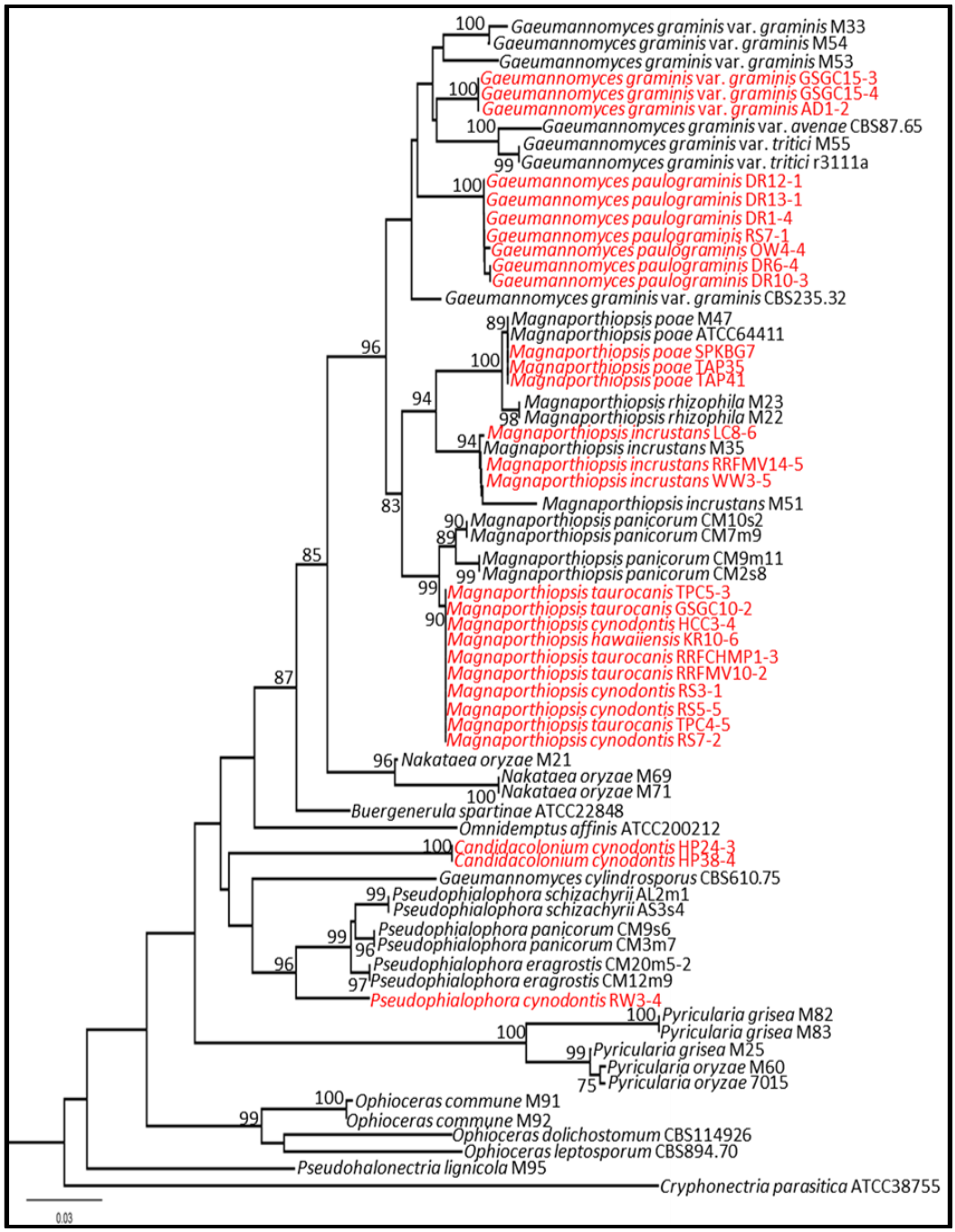


Figure B.94 Maximum Likelihood phylogram based on the minichromosome maintenance complex component 7 (*MCM7*) gene dataset. .

ML bootstrap values ≥ 75% are presented above internodes. Sequences of isolates displayed in red were generated from this study. All other data were obtained from public databases. *Cryphonectria parasitica* was chosen as the outgroup taxon.

	G. graminis var. *avenae* CBS87.65	*G. graminis* var. *graminis* M33	*G. graminis* var. *graminis* AD1-2	*G.graminis* var. *graminis* M53	*G. graminis* var. *graminis* M54	*G. graminis* var. *graminis* GSGC15-3	*G. graminis* var. *graminis* GSGC15-4	*G. graminis* var. *graminis* CBS235.32	*G.graminis* var. *tritici* M55	*G.graminis* var. *tritici* r3111a	*G. paulograminis* DR10-3	*G. paulograminis* DR12-1	*G. paulograminis* DR13-1	*G. paulograminis* DR1-4	*G. paulograminis* DR6-4	*G. paulograminis* OW4-4	*G. paulograminis* RS7-1
G. graminis var. *avenae* CBS87.65																	
G. graminis var. *graminis* M33	0.065																
G. graminis var. *graminis* AD1-2	0.051	0.043															
G.graminis var. *graminis* M53	0.055	0.043	0.039														
G. graminis var. *graminis* M54	0.059	0.006	0.037	0.037													
G. graminis var. *graminis* GSGC15-3	0.051	0.043	0.000	0.039	0.037												
G. graminis var. *graminis* GSGC15-4	0.051	0.043	0.000	0.039	0.037	0.000											
G. graminis var. *graminis* CBS235.32	0.055	0.047	0.028	0.037	0.041	0.028	0.028										
G.graminis var. *tritici* M55	0.022	0.057	0.045	0.051	0.051	0.045	0.045	0.049									
G.graminis var. *tritici* r3111a	0.022	0.057	0.045	0.051	0.051	0.045	0.045	0.049	0.000								
G. paulograminis DR10-3	0.067	0.057	0.043	0.053	0.051	0.043	0.043	0.043	0.061	0.061							
G. paulograminis DR12-1	0.065	0.055	0.041	0.051	0.049	0.041	0.041	0.041	0.059	0.059	0.002						
G. paulograminis DR13-1	0.065	0.055	0.041	0.051	0.049	0.041	0.041	0.041	0.059	0.059	0.002	0.000					
G. paulograminis DR1-4	0.065	0.055	0.041	0.051	0.049	0.041	0.041	0.041	0.059	0.059	0.002	0.000	0.000				
G. paulograminis DR6-4	0.067	0.057	0.043	0.053	0.051	0.043	0.043	0.043	0.061	0.061	0.000	0.002	0.002	0.002			
G. paulograminis OW4-4	0.067	0.057	0.043	0.053	0.051	0.043	0.043	0.043	0.061	0.061	0.004	0.002	0.002	0.002	0.004		
G. paulograminis RS7-1	0.065	0.055	0.041	0.051	0.049	0.041	0.041	0.041	0.059	0.059	0.002	0.000	0.000	0.000	0.002	0.002	

Figure B.95 Estimates of evolutionary divergence between sequences for the minichromosome maintenance complex component 7 (*MCM7*) gene of species of *Gaeumannomyces*.

	M. hawaiiensis KR10-6	*M. cynodontis* RS3-1	*M. cynodontis* RS5-5	*M. cynodontis* RS7-2	*M. incrustans* WW3-5	*M. incrustans* LC8-6	*M. incrustans* M35	*M. incrustans* M51	*M. incrustans* RRFMV14-5	*M. panicorum* CM2s8	*M. panicorum* CM7m9	*M. panicorum* CM9m11	*M. panicorum* CM10s2	*M. poae* M47	*M. poae* ATCC64411	*M. poae* TAP41	*M. poae* TAP35	*M. poae* SPKBG7	*M. rhizophila* M22	*M. rhizophila* M23	*M. taurocanis* GSGC10-2	*M. taurocanis* RRFCHMP1-3	*M. taurocanis* RRFMV10-2	*M. taurocanis* TPC5-3	*M. taurocanis* TPC4-5
M. hawaiiensis KR10-6																									
M. cynodontis RS3-1	0.000																								
M. cynodontis RS5-5	0.000	0.000																							
M. cynodontis RS7-2	0.000	0.000	0.000																						
M. incrustans WW3-5	0.044	0.044	0.044	0.044																					
M. incrustans LC8-6	0.046	0.046	0.046	0.046	0.002																				
M. incrustans M35	0.044	0.044	0.044	0.044	0.000	0.002																			
M. incrustans M51	0.065	0.065	0.065	0.065	0.020	0.022	0.020																		
M. incrustans RRFMV14-5	0.044	0.044	0.044	0.044	0.000	0.002	0.000	0.020																	
M. panicorum CM2s8	0.018	0.018	0.018	0.018	0.054	0.056	0.054	0.075	0.054																
M. panicorum CM7m9	0.014	0.014	0.014	0.014	0.046	0.048	0.046	0.067	0.046	0.012															
M. panicorum CM9m11	0.018	0.018	0.018	0.018	0.054	0.056	0.054	0.075	0.054	0.000	0.012														
M. panicorum CM10s2	0.014	0.014	0.014	0.014	0.046	0.048	0.046	0.067	0.046	0.012	0.000	0.012													
M. poae M47	0.054	0.054	0.054	0.054	0.040	0.042	0.040	0.050	0.040	0.065	0.056	0.065	0.056												
M. poae ATCC64411	0.054	0.054	0.054	0.054	0.040	0.042	0.040	0.050	0.040	0.065	0.056	0.065	0.056	0.000											
M. poae TAP41	0.054	0.054	0.054	0.054	0.040	0.042	0.040	0.050	0.040	0.065	0.056	0.065	0.056	0.000	0.000										
M. poae TAP35	0.054	0.054	0.054	0.054	0.040	0.042	0.040	0.050	0.040	0.065	0.056	0.065	0.056	0.000	0.000	0.000									
M. poae SPKBG7	0.054	0.054	0.054	0.054	0.040	0.042	0.040	0.050	0.040	0.065	0.056	0.065	0.056	0.000	0.000	0.000	0.000								
M. rhizophila M22	0.056	0.056	0.056	0.056	0.042	0.044	0.042	0.050	0.042	0.071	0.063	0.071	0.063	0.008	0.008	0.008	0.008	0.008							
M. rhizophila M23	0.056	0.056	0.056	0.056	0.042	0.044	0.042	0.050	0.042	0.071	0.063	0.071	0.063	0.008	0.008	0.008	0.008	0.008	0.000						
M. taurocanis GSGC10-2	0.000	0.000	0.000	0.000	0.044	0.046	0.044	0.065	0.044	0.018	0.014	0.018	0.014	0.054	0.054	0.054	0.054	0.054	0.056	0.056					
M. taurocanis RRFCHMP1-3	0.000	0.000	0.000	0.000	0.044	0.046	0.044	0.065	0.044	0.018	0.014	0.018	0.014	0.054	0.054	0.054	0.054	0.054	0.056	0.056	0.000				
M. taurocanis RRFMV10-2	0.000	0.000	0.000	0.000	0.044	0.046	0.044	0.065	0.044	0.018	0.014	0.018	0.014	0.054	0.054	0.054	0.054	0.054	0.056	0.056	0.000	0.000			
M. taurocanis TPC5-3	0.000	0.000	0.000	0.000	0.044	0.046	0.044	0.065	0.044	0.018	0.014	0.018	0.014	0.054	0.054	0.054	0.054	0.054	0.056	0.056	0.000	0.000	0.000		
M. taurocanis TPC4-5	0.000	0.000	0.000	0.000	0.044	0.046	0.044	0.065	0.044	0.018	0.014	0.018	0.014	0.054	0.054	0.054	0.054	0.054	0.056	0.056	0.000	0.000	0.000	0.000	

Figure B.96 Estimates of evolutionary divergence between sequences for the minichromosome maintenance complex component 7 (*MCM7*) gene of species of *Magnaporthiopsis*.

	P. cynodontis RW3-4	*P. eragrostis* CM12m9	*P. eragrostis* CM20m5-2	*P. panicorum* CM3m7	*P. panicorum* CM9s6	*P. schizachyrii* AL3s4	*P. schizachyrii* AL2m1
P. cynodontis RW3-4							
P. eragrostis CM12m9	0.052						
P. eragrostis CM20m5-2	0.049	0.003					
P. panicorum CM3m7	0.051	0.019	0.015				
P. panicorum CM9s6	0.051	0.019	0.015	0.000			
P. schizachyrii AL3s4	0.056	0.022	0.020	0.022	0.022		
P. schizachyrii AL2m1	0.056	0.022	0.020	0.022	0.022	0.000	

Figure B.97 Estimates of evolutionary divergence between sequences for the minichromosome maintenance complex component 7 (*MCM7*) gene of species of *Pseudophialophora*.

Table B.7 Maximum likelihood fits of nucleotide substitution models for the translation elongation factor 1-alpha gene (*TEF1*) gene dataset.

Model	**BIC**[†]
Tamura-Nei (TN93)+G	7736.09
TN93+G+I	7741.69
General Time Reversible (GTR) + G	7759.31
GTR+G+I	7765.04
Tamura 3-parameter (T92) + G	7787.37
TN93+I	7789.57
T92+G+I	7794.57
Hasegawa-Kishino-Yano (HKY)+G	7795.91
HKY+G+I	7804.05
GTR+I	7811.67
Kimura 2-parameter (K2) + G	7820.52
K2+G+I	7826.11
T92+I	7831.11
HKY+I	7841.02
Jukes-Cantor (JC)+G	7923.87
JC+G+I	7928.91
JC+I	7957.37
TN93	8209.88
GTR	8228.20
T92	8290.93
K2	8295.29
K2+I	8305.81
HKY	8311.05
JC	8386.98

[†] Bayesian Information Criterion scores, model with lowest score most adequately describes the substitution pattern.
[§] Gamma distribution.
[¶] Invariable rates among sites.

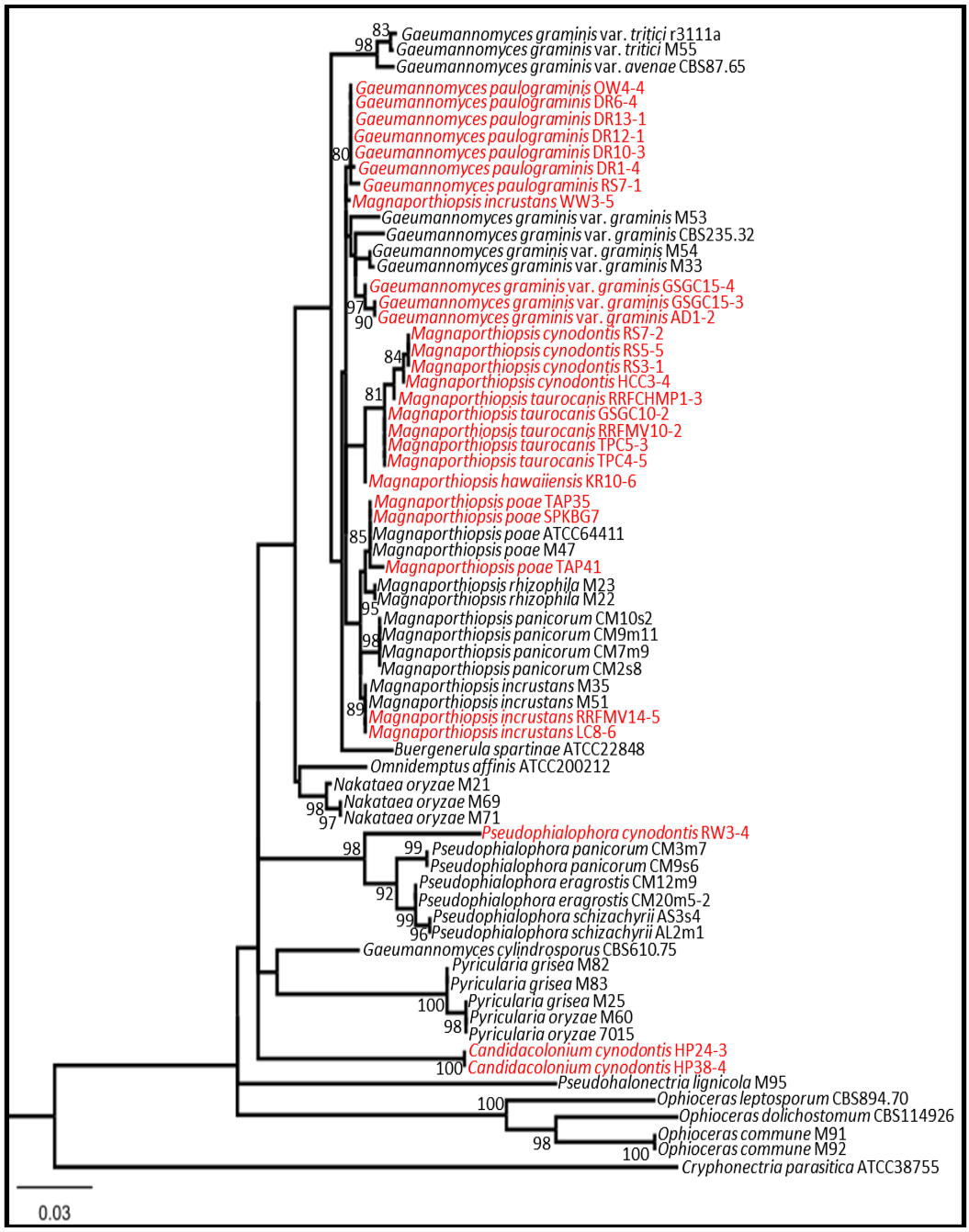
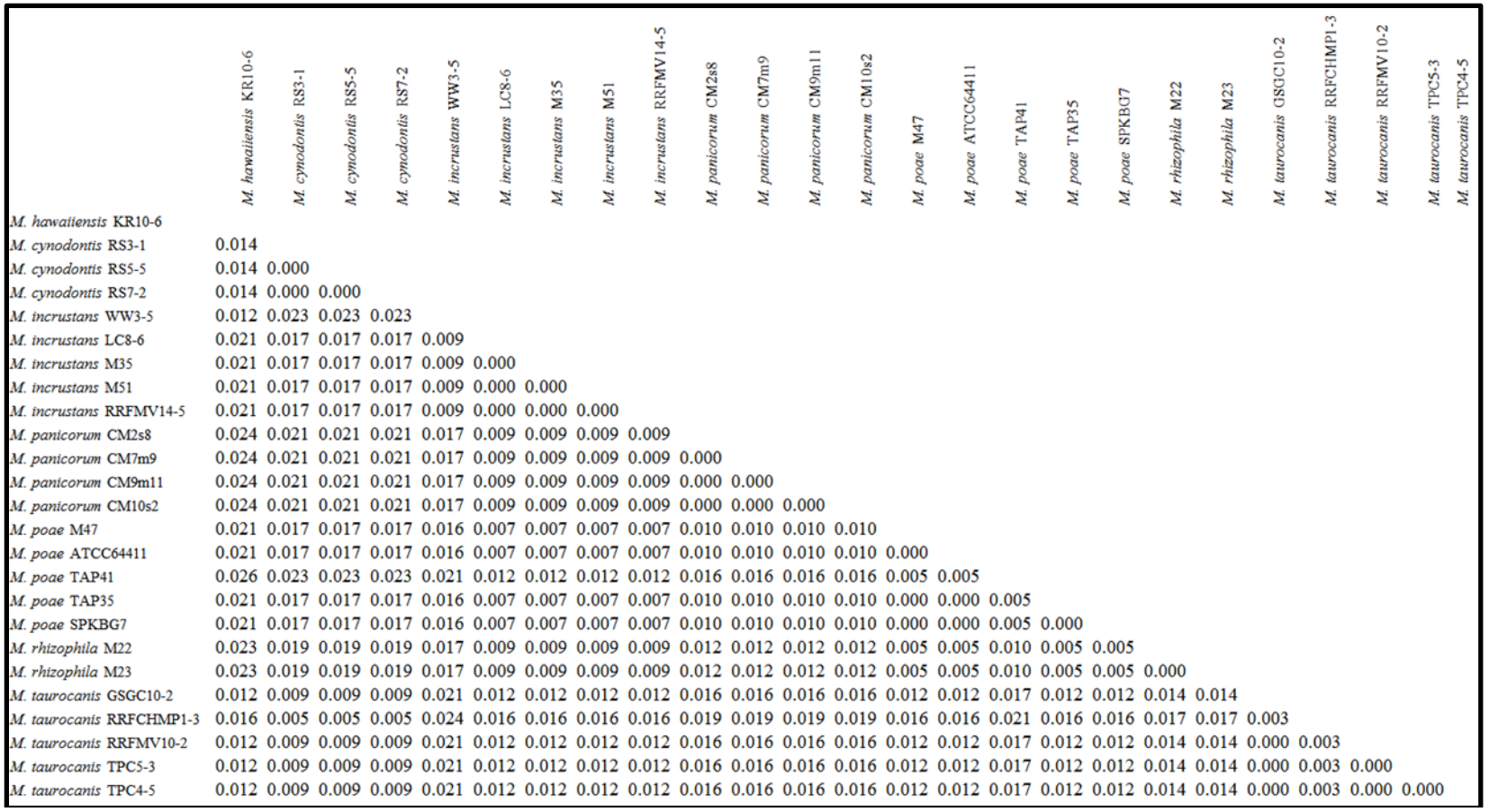


Figure B.98 Maximum Likelihood phylogram based on the translation elongation factor 1-alpha gene (*TEF1*) gene dataset.

ML bootstrap values ≥ 75% are presented above internodes. Sequences of isolates displayed in red were generated from this study. All other data were obtained from public databases. *Cryphonectria parasitica* was chosen as the outgroup taxon.

	G. graminis var. *avenae* CBS87.65	*G. graminis* var. *graminis* M33	*G. graminis* var. *graminis* AD1-2	*G.graminis* var. *graminis* M53	*G. graminis* var. *graminis* M54	*G. graminis* var. *graminis* GSGC15-3	*G. graminis* var. *graminis* GSGC15-4	*G. graminis* var. *graminis* CBS235.32	*G.graminis* var. *tritici* M55	*G.graminis* var. *tritici* r3111a	*G. paulograminis* DR10-3	*G. paulograminis* DR12-1	*G. paulograminis* DR13-1	*G. paulograminis* DR1-4	*G. paulograminis* DR6-4	*G. paulograminis* OW4-4	*G. paulograminis* RS7-1
G. graminis var. *avenae* CBS87.65																	
G. graminis var. *graminis* M33	0.049																
G. graminis var. *graminis* AD1-2	0.043	0.022															
G.graminis var. *graminis* M53	0.049	0.021	0.025														
G. graminis var. *graminis* M54	0.044	0.004	0.018	0.017													
G. graminis var. *graminis* GSGC15-3	0.043	0.022	0.000	0.025	0.018												
G. graminis var. *graminis* GSGC15-4	0.042	0.018	0.004	0.021	0.014	0.004											
G. graminis var. *graminis* CBS235.32	0.043	0.028	0.022	0.031	0.024	0.022	0.021										
G.graminis var. *tritici* M55	0.011	0.043	0.038	0.046	0.039	0.038	0.036	0.038									
G.graminis var. *tritici* r3111a	0.011	0.046	0.040	0.049	0.042	0.040	0.039	0.040	0.003								
G. paulograminis DR10-3	0.044	0.028	0.029	0.026	0.024	0.029	0.025	0.032	0.042	0.044							
G. paulograminis DR12-1	0.044	0.028	0.029	0.026	0.024	0.029	0.025	0.032	0.042	0.044	0.000						
G. paulograminis DR13-1	0.044	0.028	0.029	0.026	0.024	0.029	0.025	0.032	0.042	0.044	0.000	0.000					
G. paulograminis DR1-4	0.044	0.028	0.029	0.026	0.024	0.029	0.025	0.032	0.042	0.044	0.003	0.003	0.003				
G. paulograminis DR6-4	0.044	0.028	0.029	0.026	0.024	0.029	0.025	0.032	0.042	0.044	0.000	0.000	0.000	0.003			
G. paulograminis OW4-4	0.044	0.028	0.029	0.026	0.024	0.029	0.025	0.032	0.042	0.044	0.000	0.000	0.000	0.003	0.000		
G. paulograminis RS7-1	0.047	0.031	0.032	0.029	0.026	0.032	0.028	0.035	0.044	0.047	0.003	0.003	0.003	0.006	0.003	0.003	

Figure B.99 Estimates of evolutionary divergence between sequences for the translation elongation factor 1-alpha gene (*TEF1*) gene of species of *Gaeumannomyces*.



	M. hawaiiensis KR10-6	*M. cynodontis* RS3-1	*M. cynodontis* RS5-5	*M. cynodontis* RS7-2	*M. incrustans* WW3-5	*M. incrustans* LC8-6	*M. incrustans* M35	*M. incrustans* M51	*M. incrustans* RRFMV14-5	*M. panicorum* CM2s8	*M. panicorum* CM7m9	*M. panicorum* CM9m11	*M. panicorum* CM10s2	*M. poae* M47	*M. poae* ATCC64411	*M. poae* TAP41	*M. poae* TAP35	*M. poae* SPKBG7	*M. rhizophila* M22	*M. rhizophila* M23	*M. taurocanis* GSGC10-2	*M. taurocanis* RRFCHMP1-3	*M. taurocanis* RRFMV10-2	*M. taurocanis* TPC5-3	*M. taurocanis* TPC4-5
M. hawaiiensis KR10-6																									
M. cynodontis RS3-1	0.014																								
M. cynodontis RS5-5	0.014	0.000																							
M. cynodontis RS7-2	0.014	0.000	0.000																						
M. incrustans WW3-5	0.012	0.023	0.023	0.023																					
M. incrustans LC8-6	0.021	0.017	0.017	0.017	0.009																				
M. incrustans M35	0.021	0.017	0.017	0.017	0.009	0.000																			
M. incrustans M51	0.021	0.017	0.017	0.017	0.009	0.000	0.000																		
M. incrustans RRFMV14-5	0.021	0.017	0.017	0.017	0.009	0.000	0.000	0.000																	
M. panicorum CM2s8	0.024	0.021	0.021	0.021	0.017	0.009	0.009	0.009	0.009																
M. panicorum CM7m9	0.024	0.021	0.021	0.021	0.017	0.009	0.009	0.009	0.009	0.000															
M. panicorum CM9m11	0.024	0.021	0.021	0.021	0.017	0.009	0.009	0.009	0.009	0.000	0.000														
M. panicorum CM10s2	0.024	0.021	0.021	0.021	0.017	0.009	0.009	0.009	0.009	0.000	0.000	0.000													
M. poae M47	0.021	0.017	0.017	0.017	0.016	0.007	0.007	0.007	0.007	0.010	0.010	0.010	0.010												
M. poae ATCC64411	0.021	0.017	0.017	0.017	0.016	0.007	0.007	0.007	0.007	0.010	0.010	0.010	0.010	0.000											
M. poae TAP41	0.026	0.023	0.023	0.023	0.021	0.012	0.012	0.012	0.012	0.016	0.016	0.016	0.016	0.005	0.005										
M. poae TAP35	0.021	0.017	0.017	0.017	0.016	0.007	0.007	0.007	0.007	0.010	0.010	0.010	0.010	0.000	0.000	0.005									
M. poae SPKBG7	0.021	0.017	0.017	0.017	0.016	0.007	0.007	0.007	0.007	0.010	0.010	0.010	0.010	0.000	0.000	0.005	0.000								
M. rhizophila M22	0.023	0.019	0.019	0.019	0.017	0.009	0.009	0.009	0.009	0.012	0.012	0.012	0.012	0.005	0.005	0.010	0.005	0.005							
M. rhizophila M23	0.023	0.019	0.019	0.019	0.017	0.009	0.009	0.009	0.009	0.012	0.012	0.012	0.012	0.005	0.005	0.010	0.005	0.005	0.000						
M. taurocanis GSGC10-2	0.012	0.009	0.009	0.009	0.021	0.012	0.012	0.012	0.012	0.016	0.016	0.016	0.016	0.012	0.012	0.017	0.012	0.012	0.014	0.014					
M. taurocanis RRFCHMP1-3	0.016	0.005	0.005	0.005	0.024	0.016	0.016	0.016	0.016	0.019	0.019	0.019	0.019	0.016	0.016	0.021	0.016	0.016	0.017	0.017	0.003				
M. taurocanis RRFMV10-2	0.012	0.009	0.009	0.009	0.021	0.012	0.012	0.012	0.012	0.016	0.016	0.016	0.016	0.012	0.012	0.017	0.012	0.012	0.014	0.014	0.000	0.003			
M. taurocanis TPC5-3	0.012	0.009	0.009	0.009	0.021	0.012	0.012	0.012	0.012	0.016	0.016	0.016	0.016	0.012	0.012	0.017	0.012	0.012	0.014	0.014	0.000	0.003	0.000		
M. taurocanis TPC4-5	0.012	0.009	0.009	0.009	0.021	0.012	0.012	0.012	0.012	0.016	0.016	0.016	0.016	0.012	0.012	0.017	0.012	0.012	0.014	0.014	0.000	0.003	0.000	0.000	

Figure B.100 Estimates of evolutionary divergence between sequences for the translation elongation factor 1-alpha gene (*TEF1*) gene of species of *Magnaporthiopsis*.

	P. cynodontis RW3-4	*P. eragrostis* CM12m9	*P. eragrostis* CM20m5-2	*P. panicorum* CM3m7	*P. panicorum* CM9s6	*P. schizachyrii* AL3s4	*P. schizachyrii* AL2m1
P. cynodontis RW3-4							
P. eragrostis CM12m9	0.048						
P. eragrostis CM20m5-2	0.048	0.000					
P. panicorum CM3m7	0.045	0.023	0.023				
P. panicorum CM9s6	0.045	0.023	0.023	0.000			
P. schizachyrii AL3s4	0.048	0.010	0.010	0.024	0.024		
P. schizachyrii AL2m1	0.047	0.009	0.009	0.023	0.023	0.001	

Figure B.101 Estimates of evolutionary divergence between sequences for the translation elongation factor 1-alpha gene (*TEF1*) gene of species of *Pseudophialophora*.

Table B.8 Maximum likelihood fits of nucleotide substitution models for the largest subunit of the largest subunit of RNA polymerase II (RPBI) gene dataset.

Model	BIC[†]
Tamura-Nei (TN93)+G+I	12764.57
TN93+G	12767.23
General Time Reversible (GTR) + G + I	12788.25
GTR+G	12790.57
Tamura 3-parameter (T92) + G	12796.75
T92+G+I	12801.42
Kimura 2-parameter (K2) + G	12835.04
K2+G+I	12838.42
Hasegawa-Kishino-Yano (HKY)+G	12839.86
HKY+G+I	12844.02
TN93+I	12908.04
GTR+I	12936.08
T92+I	12980.10
K2+I	12994.49
HKY+I	13023.42
Jukes-Cantor (JC)+G	13102.47
JC+G+I	13103.46
JC+I	13237.35
TN93	13530.98
GTR	13555.55
K2	13573.65
T92	13604.76
HKY	13661.41
JC	13794.64

[†] Bayesian Information Criterion scores, model with lowest score most adequately describes the substitution pattern.
[§] Gamma distribution.
[¶] Invariable rates among sites.

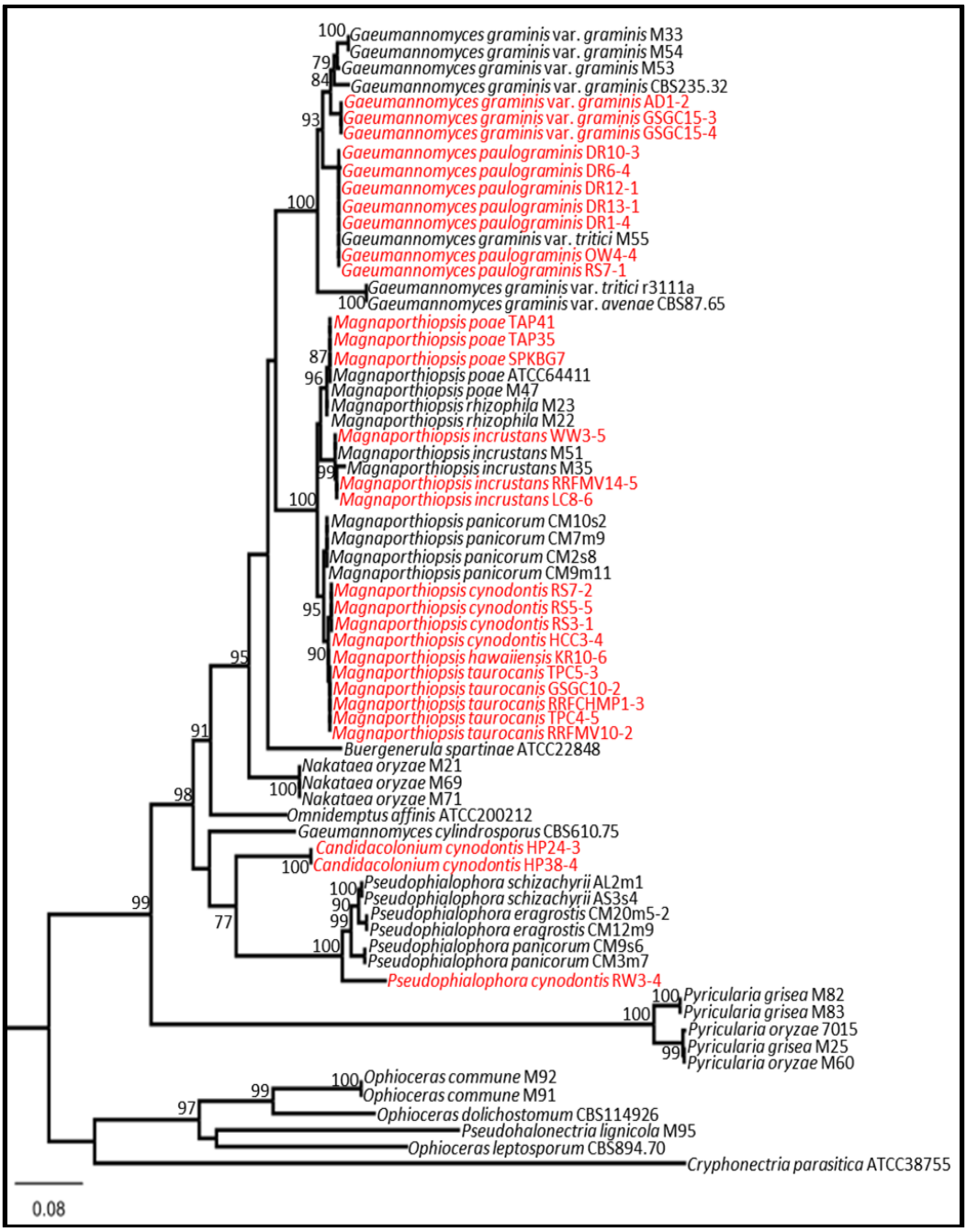
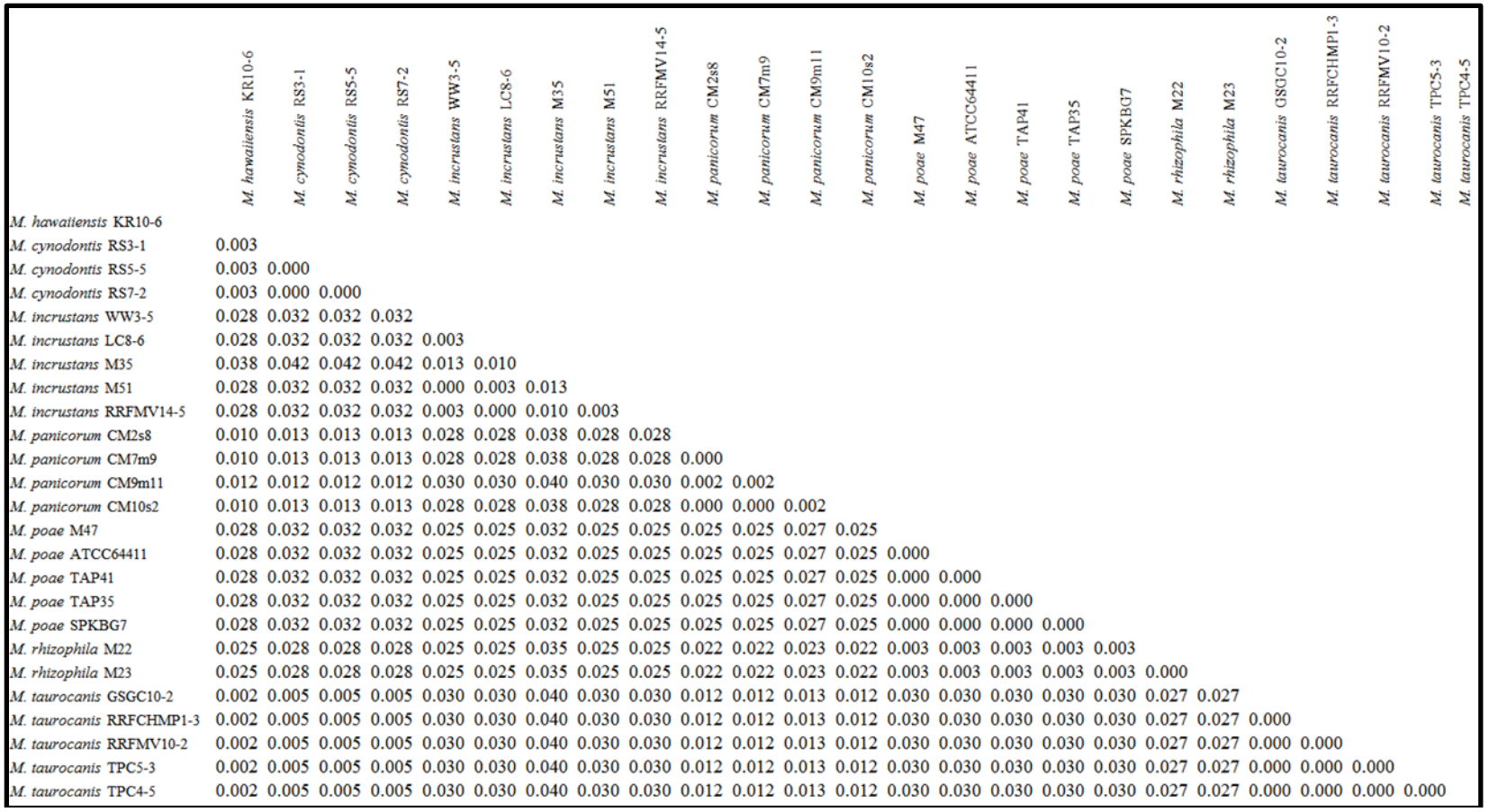


Figure B.102 Maximum Likelihood phylogram based on the largest subunit of RNA polymerase II (*RPBI*) gene dataset.

ML bootstrap values ≥ 75% are presented above internodes. Sequences of isolates displayed in red were generated from this study. All other data were obtained from public databases. *Cryphonectria parasitica* was chosen as the outgroup taxon.

	G. graminis var. *avenae* CBS87.65	*G. graminis* var. *graminis* M33	*G. graminis* var. *graminis* AD1-2	*G.graminis* var. *graminis* M53	*G. graminis* var. *graminis* M54	*G. graminis* var. *graminis* GSGC15-3	*G. graminis* var. *graminis* GSGC15-4	*G. graminis* var. *graminis* CBS235.32	*G.graminis* var. *tritici* M55	*G.graminis* var. *tritici* r3111a	*G. paulograminis* DR10-3	*G. paulograminis* DR12-1	*G. paulograminis* DR13-1	*G. paulograminis* DR1-4	*G. paulograminis* DR6-4	*G. paulograminis* OW4-4	*G. paulograminis* RS7-1
G. graminis var. *avenae* CBS87.65																	
G. graminis var. *graminis* M33	0.089																
G. graminis var. *graminis* AD1-2	0.079	0.030															
G.graminis var. *graminis* M53	0.083	0.018	0.025														
G. graminis var. *graminis* M54	0.089	0.000	0.030	0.018													
G. graminis var. *graminis* GSGC15-3	0.079	0.030	0.000	0.025	0.030												
G. graminis var. *graminis* GSGC15-4	0.079	0.030	0.000	0.025	0.030	0.000											
G. graminis var. *graminis* CBS235.32	0.078	0.033	0.031	0.028	0.033	0.031	0.031										
G.graminis var. *tritici* M55	0.078	0.046	0.043	0.040	0.046	0.043	0.043	0.046									
G.graminis var. *tritici* r3111a	0.003	0.086	0.076	0.079	0.086	0.076	0.076	0.074	0.074								
G. paulograminis DR10-3	0.079	0.048	0.045	0.041	0.048	0.045	0.045	0.048	0.002	0.076							
G. paulograminis DR12-1	0.078	0.046	0.043	0.040	0.046	0.043	0.043	0.046	0.000	0.074	0.002						
G. paulograminis DR13-1	0.078	0.046	0.043	0.040	0.046	0.043	0.043	0.046	0.000	0.074	0.002	0.000					
G. paulograminis DR1-4	0.078	0.046	0.043	0.040	0.046	0.043	0.043	0.046	0.000	0.074	0.002	0.000	0.000				
G. paulograminis DR6-4	0.083	0.051	0.048	0.045	0.051	0.048	0.048	0.051	0.005	0.079	0.007	0.005	0.005	0.005			
G. paulograminis OW4-4	0.078	0.046	0.043	0.040	0.046	0.043	0.043	0.046	0.000	0.074	0.002	0.000	0.000	0.000	0.005		
G. paulograminis RS7-1	0.078	0.046	0.043	0.040	0.046	0.043	0.043	0.046	0.000	0.074	0.002	0.000	0.000	0.000	0.005	0.000	

Figure B.103 Estimates of evolutionary divergence between sequences for the largest subunit of RNA polymerase II (RPBI) gene of species of *Gaeumannomyces*.



	M. hawaiiensis KR10-6	*M. cynodontis* RS3-1	*M. cynodontis* RS5-5	*M. cynodontis* RS7-2	*M. incrustans* WW3-5	*M. incrustans* LC8-6	*M. incrustans* M35	*M. incrustans* M51	*M. incrustans* RRFMV14-5	*M. panicorum* CM2s8	*M. panicorum* CM7m9	*M. panicorum* CM9m11	*M. panicorum* CM10s2	*M. poae* M47	*M. poae* ATCC64411	*M. poae* TAP41	*M. poae* TAP35	*M. poae* SPKBG7	*M. rhizophila* M22	*M. rhizophila* M23	*M. taurocanis* GSGC10-2	*M. taurocanis* RRFCHMP1-3	*M. taurocanis* RRFMV10-2	*M. taurocanis* TPC5-3	*M. taurocanis* TPC4-5
M. hawaiiensis KR10-6																									
M. cynodontis RS3-1	0.003																								
M. cynodontis RS5-5	0.003	0.000																							
M. cynodontis RS7-2	0.003	0.000	0.000																						
M. incrustans WW3-5	0.028	0.032	0.032	0.032																					
M. incrustans LC8-6	0.028	0.032	0.032	0.032	0.003																				
M. incrustans M35	0.038	0.042	0.042	0.042	0.013	0.010																			
M. incrustans M51	0.028	0.032	0.032	0.032	0.000	0.003	0.013																		
M. incrustans RRFMV14-5	0.028	0.032	0.032	0.032	0.003	0.000	0.010	0.003																	
M. panicorum CM2s8	0.010	0.013	0.013	0.013	0.028	0.028	0.038	0.028	0.028																
M. panicorum CM7m9	0.010	0.013	0.013	0.013	0.028	0.028	0.038	0.028	0.028	0.000															
M. panicorum CM9m11	0.012	0.012	0.012	0.012	0.030	0.030	0.040	0.030	0.030	0.002	0.002														
M. panicorum CM10s2	0.010	0.013	0.013	0.013	0.028	0.028	0.038	0.028	0.028	0.000	0.000	0.002													
M. poae M47	0.028	0.032	0.032	0.032	0.025	0.025	0.032	0.025	0.025	0.025	0.025	0.027	0.025												
M. poae ATCC64411	0.028	0.032	0.032	0.032	0.025	0.025	0.032	0.025	0.025	0.025	0.025	0.027	0.025	0.000											
M. poae TAP41	0.028	0.032	0.032	0.032	0.025	0.025	0.032	0.025	0.025	0.025	0.025	0.027	0.025	0.000	0.000										
M. poae TAP35	0.028	0.032	0.032	0.032	0.025	0.025	0.032	0.025	0.025	0.025	0.025	0.027	0.025	0.000	0.000	0.000									
M. poae SPKBG7	0.028	0.032	0.032	0.032	0.025	0.025	0.032	0.025	0.025	0.025	0.025	0.027	0.025	0.000	0.000	0.000	0.000								
M. rhizophila M22	0.025	0.028	0.028	0.028	0.025	0.025	0.035	0.025	0.025	0.022	0.022	0.023	0.022	0.003	0.003	0.003	0.003	0.003							
M. rhizophila M23	0.025	0.028	0.028	0.028	0.025	0.025	0.035	0.025	0.025	0.022	0.022	0.023	0.022	0.003	0.003	0.003	0.003	0.003	0.000						
M. taurocanis GSGC10-2	0.002	0.005	0.005	0.005	0.030	0.030	0.040	0.030	0.030	0.012	0.012	0.013	0.012	0.030	0.030	0.030	0.030	0.030	0.027	0.027					
M. taurocanis RRFCHMP1-3	0.002	0.005	0.005	0.005	0.030	0.030	0.040	0.030	0.030	0.012	0.012	0.013	0.012	0.030	0.030	0.030	0.030	0.030	0.027	0.027	0.000				
M. taurocanis RRFMV10-2	0.002	0.005	0.005	0.005	0.030	0.030	0.040	0.030	0.030	0.012	0.012	0.013	0.012	0.030	0.030	0.030	0.030	0.030	0.027	0.027	0.000	0.000			
M. taurocanis TPC5-3	0.002	0.005	0.005	0.005	0.030	0.030	0.040	0.030	0.030	0.012	0.012	0.013	0.012	0.030	0.030	0.030	0.030	0.030	0.027	0.027	0.000	0.000	0.000		
M. taurocanis TPC4-5	0.002	0.005	0.005	0.005	0.030	0.030	0.040	0.030	0.030	0.012	0.012	0.013	0.012	0.030	0.030	0.030	0.030	0.030	0.027	0.027	0.000	0.000	0.000	0.000	

Figure B.104 Estimates of evolutionary divergence between sequences for the largest subunit of RNA polymerase II (RPBI) gene of species of *Magnaporthiopsis*.

	P. cynodontis RW3-4	*P. eragrostis* CM12m9	*P. eragrostis* CM20m5-2	*P. panicorum* CM3m7	*P. panicorum* CM9s6	*P. schizachyrii* AL3s4	*P. schizachyrii* AL2m1
P. cynodontis RW3-4							
P. eragrostis CM12m9	0.074						
P. eragrostis CM20m5-2	0.074	0.000					
P. panicorum CM3m7	0.074	0.029	0.029				
P. panicorum CM9s6	0.074	0.029	0.029	0.000			
P. schizachyrii AL3s4	0.070	0.012	0.012	0.023	0.023		
P. schizachyrii AL2m1	0.070	0.012	0.012	0.023	0.023	0.000	

Figure B.105 Estimates of evolutionary divergence between sequences for the largest subunit of RNA polymerase II (RPBI) gene of species of *Pseudophialophora*.

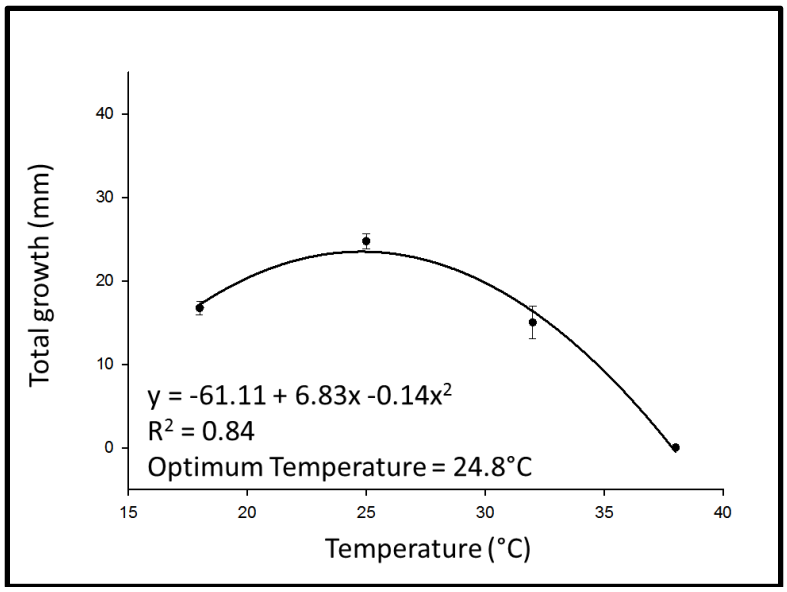


Figure B.106 Total growth of *Gaeumannomyces graminis* var. *graminis* isolate AD1–2 after 7-day incubation at a temperature range of 18 to 38 C.

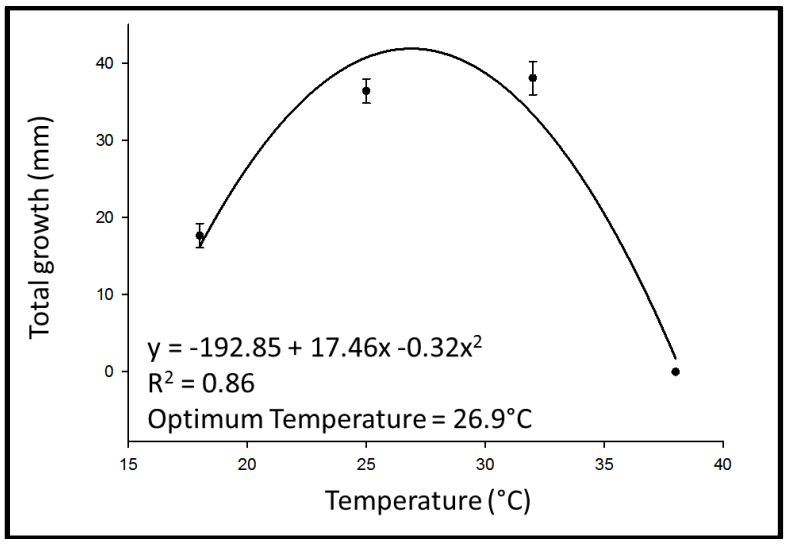


Figure B.107 Total growth of *Gaeumannomyces paulograminis* isolate DR1–4 after 7-day incubation at a temperature range of 18 to 38 C.

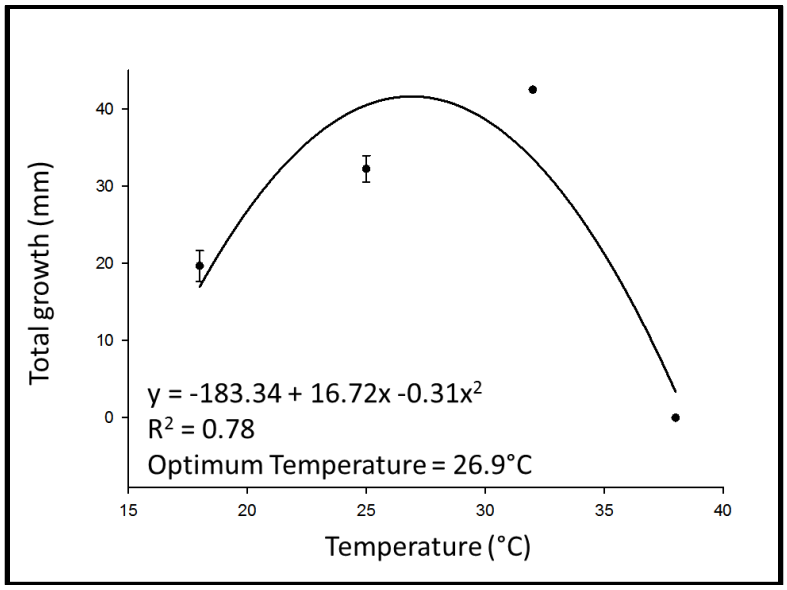


Figure B.108 Total growth of *Gaeumannomyces paulograminis* isolate DR6–4 after 7-day incubation at a temperature range of 18 to 38 C.

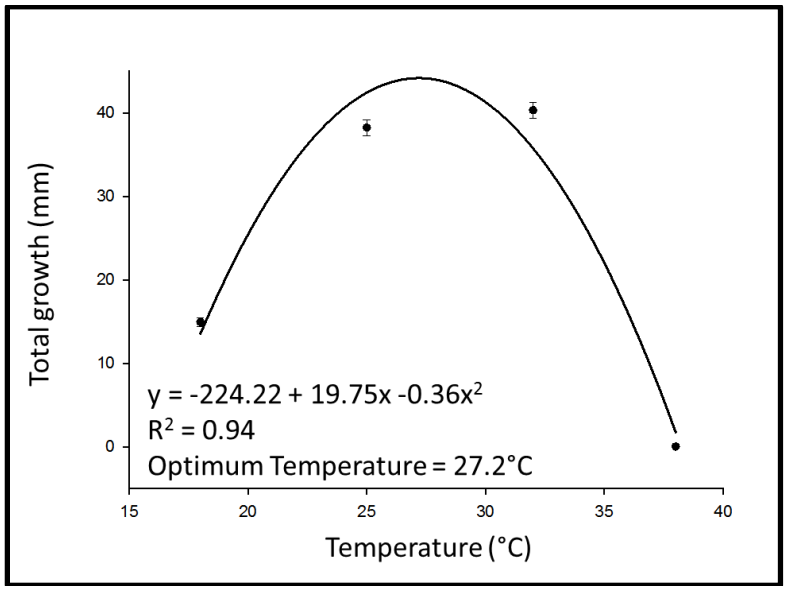


Figure B.109 Total growth of *Gaeumannomyces paulograminis* isolate DR10–3 after 7-day incubation at a temperature range of 18 to 38 C.

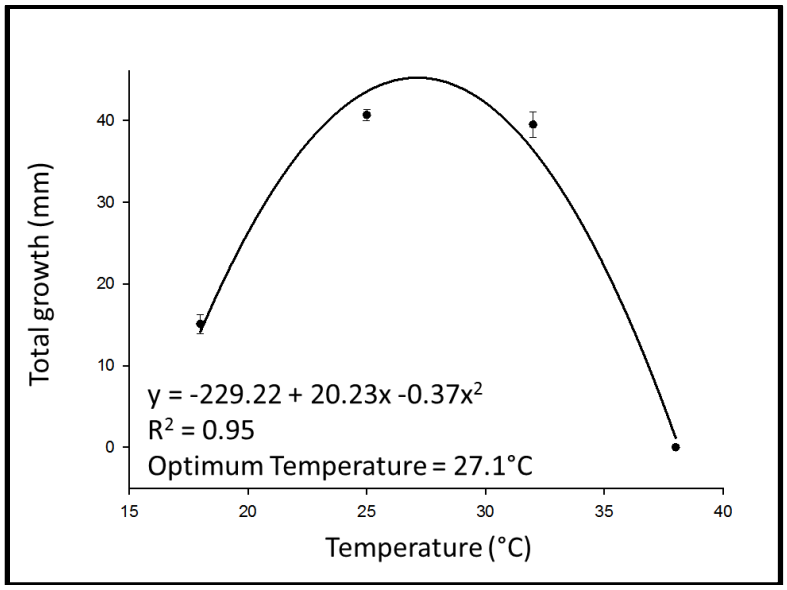


Figure B.110 Total growth of *Gaeumannomyces paulograminis* isolate DR12–1 after 7-day incubation at a temperature range of 18 to 38 C.

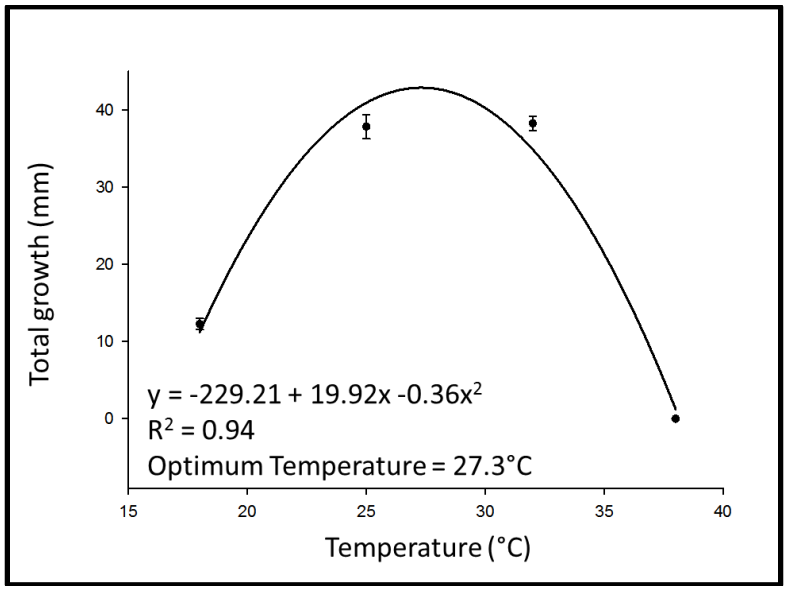


Figure B.111 Total growth of *Gaeumannomyces paulograminis* isolate DR13–1 after 7-day incubation at a temperature range of 18 to 38 C.

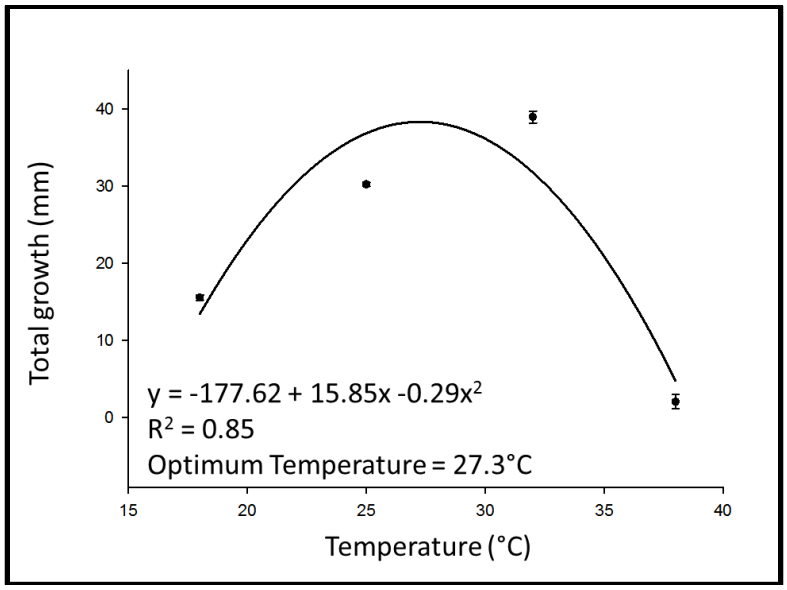


Figure B.112 Total growth of *Magnaporthiopsis taurocanis* isolate GSGC10–2 after 7-day incubation at a temperature range of 18 to 38 C.

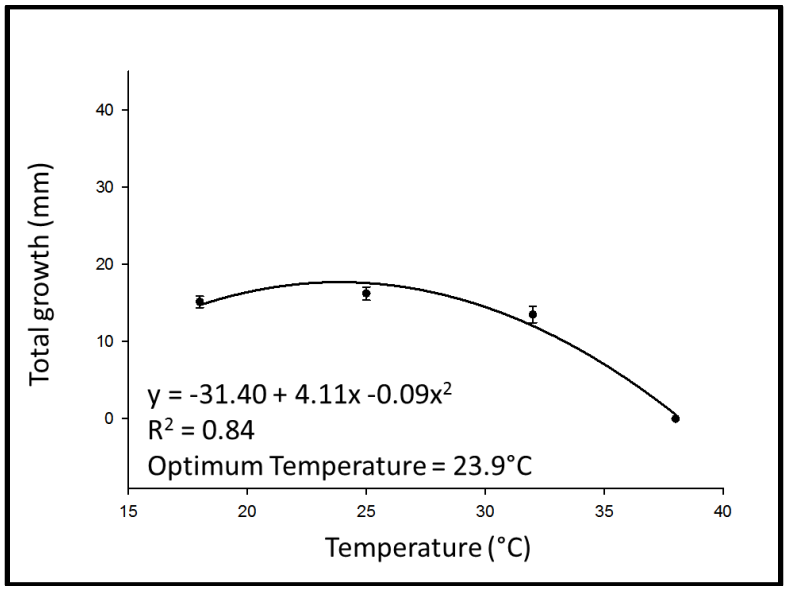


Figure B.113 Total growth of *Gaeumannomyces graminis* var. *graminis* isolate GSGC15–3 after 7-day incubation at a temperature range of 18 to 38 C.

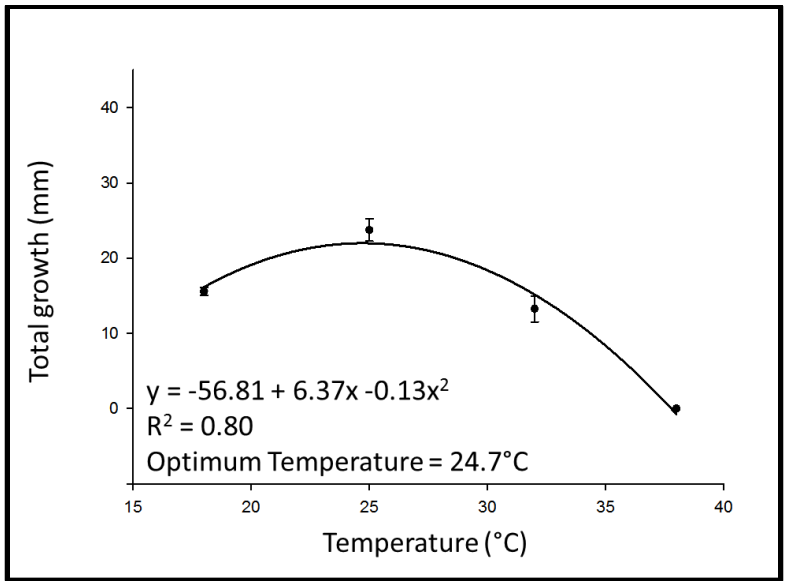


Figure B.114 Total growth of *Gaeumannomyces graminis* var. *graminis* isolate GSGC15–4 after 7-day incubation at a temperature range of 18 to 38 C.

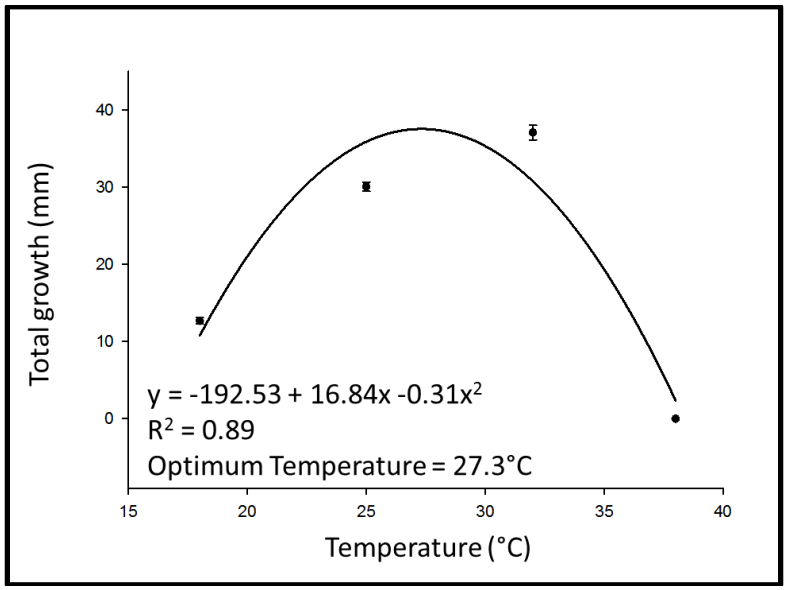


Figure B.115 Total growth of *Magnaporthiopsis cynodontis* isolate HCC3–4 after 7-day incubation at a temperature range of 18 to 38 C.

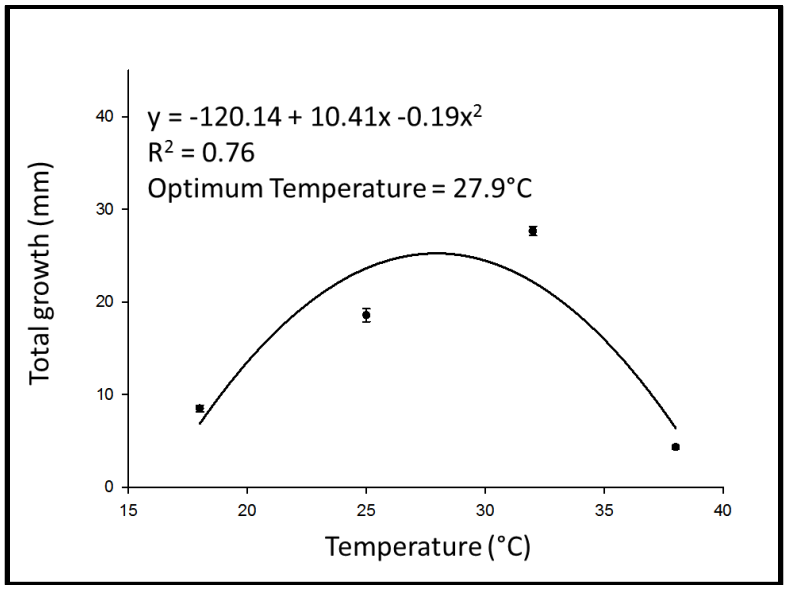


Figure B.116 Total growth of *Candidacolonium cynodontis* isolate HP24–3 after 7-day incubation at a temperature range of 18 to 38 C.

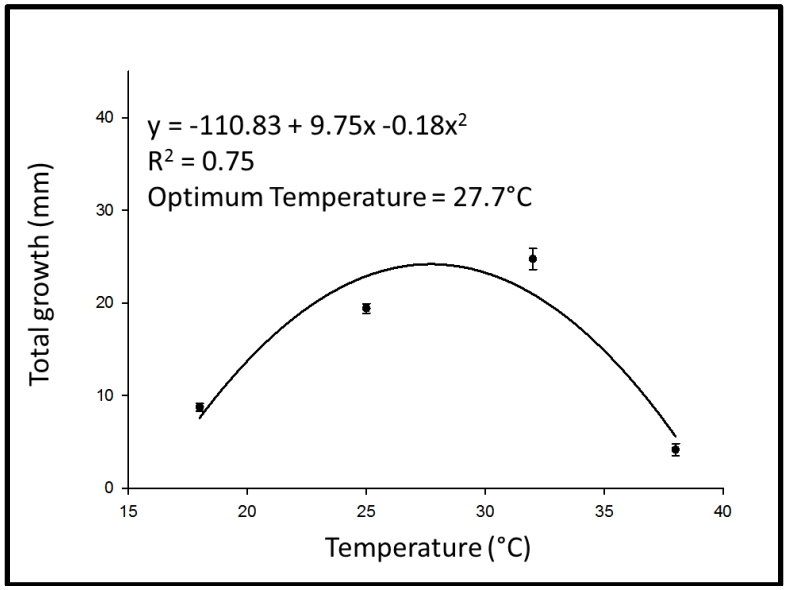


Figure B.117 Total growth of *Candidacolonium cynodontis* isolate HP38–4 after 7-day incubation at a temperature range of 18 to 38 C.

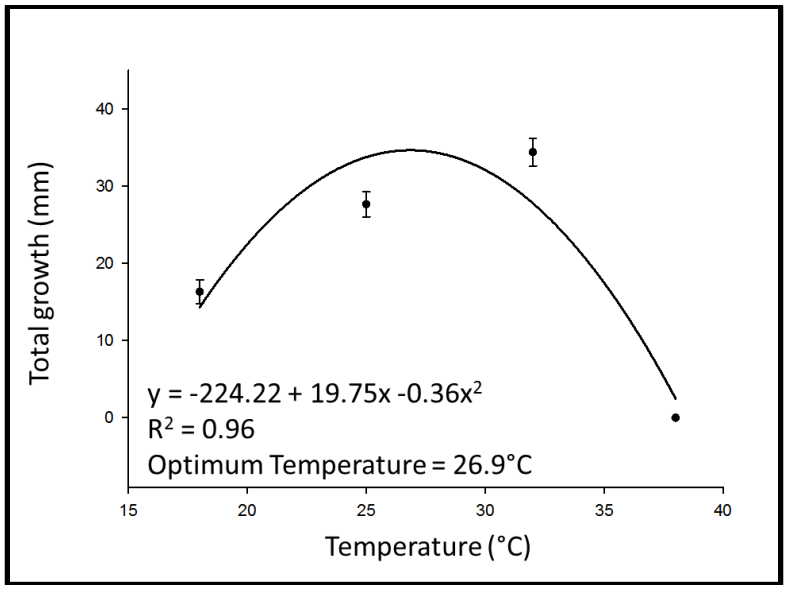


Figure B.118 Total growth of *Magnaporthiopsis hawaiiensis* isolate KR10–6 after 7-day incubation at a temperature range of 18 to 38 C.

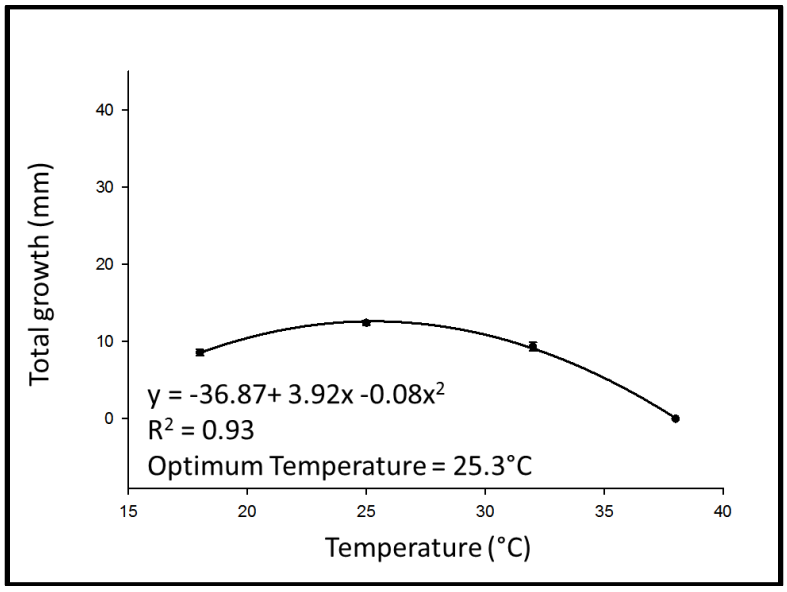


Figure B.119 Total growth of *Magnaporthiopsis incrustans* isolate LC8–6 after 7-day incubation at a temperature range of 18 to 38 C.

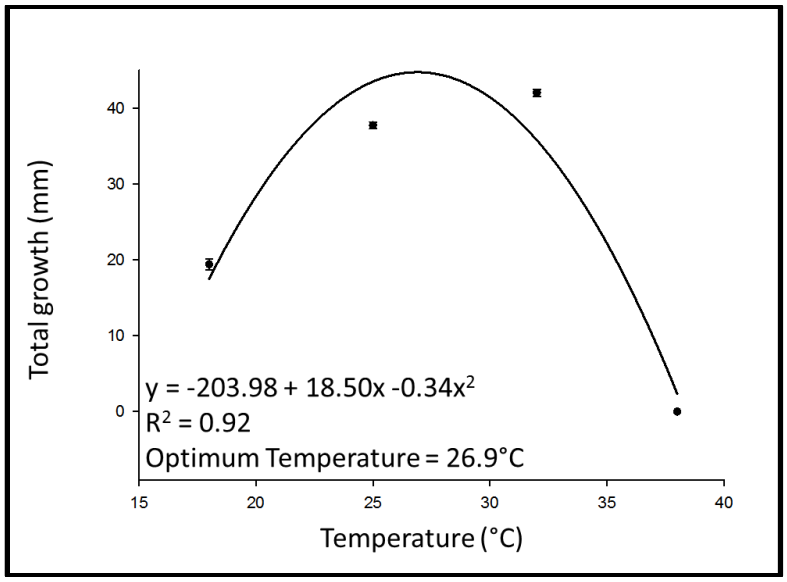


Figure B.120 Total growth of *Gaeumannomyces paulograminis* isolate OW4–4 after 7-day incubation at a temperature range of 18 to 38 C.

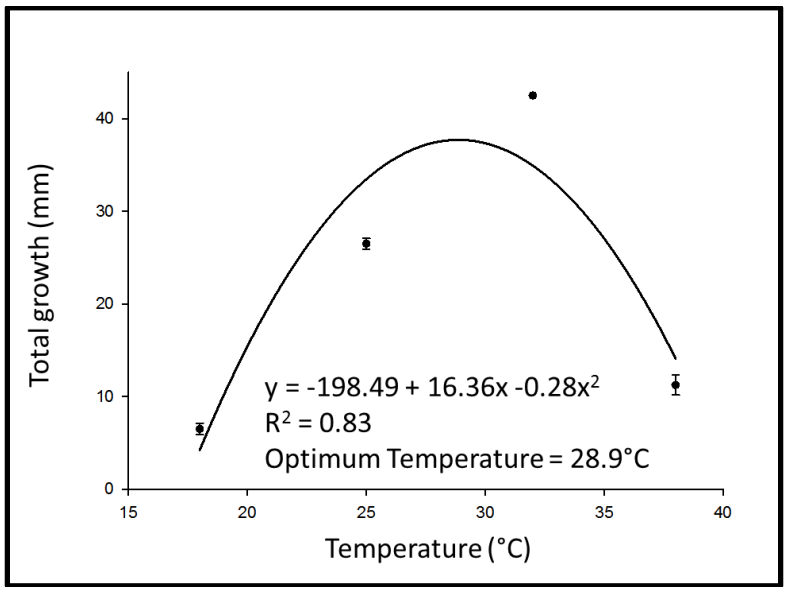


Figure B.121 Total growth of *Magnaporthiopsis taurocanis* isolate RRFCHMP1–3 after 7-day incubation at a temperature range of 18 to 38 C.

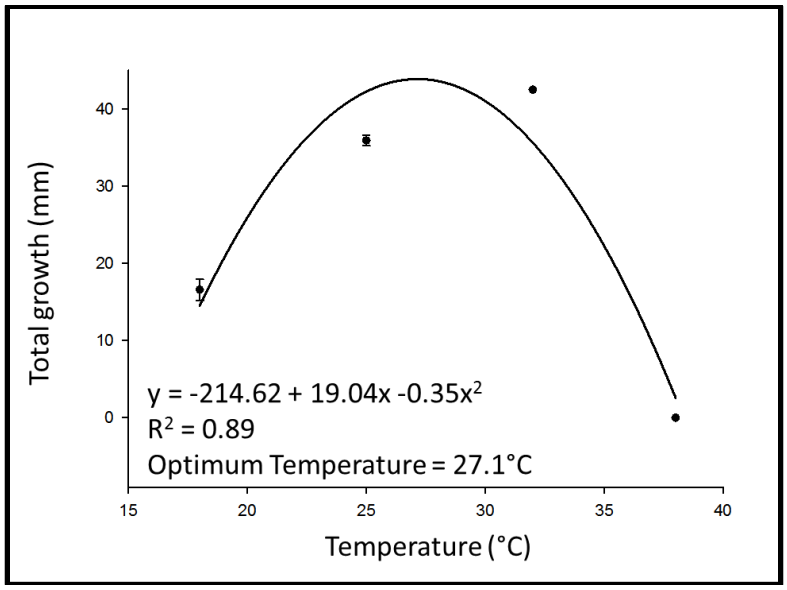


Figure B.122 Total growth of *Magnaporthiopsis taurocanis* isolate RRFMV10–2 after 7-day incubation at a temperature range of 18 to 38 C.

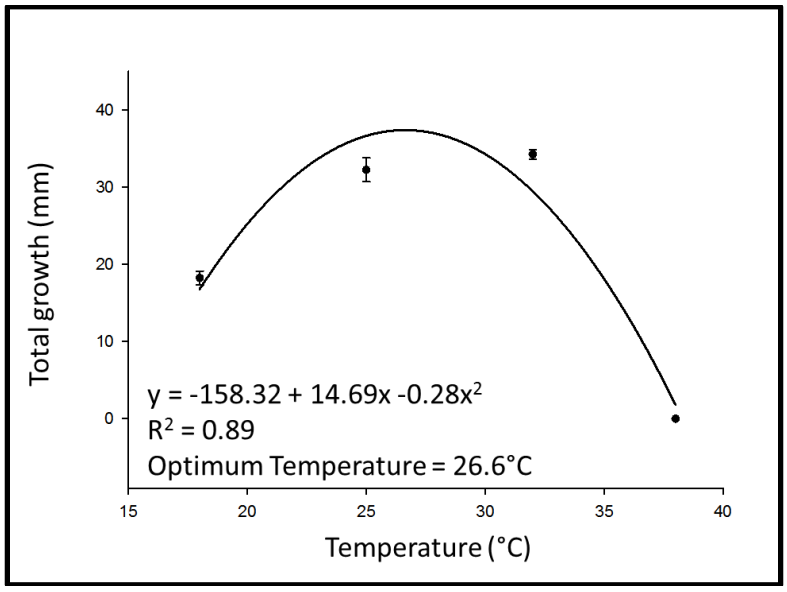


Figure B.123 Total growth of *Magnaporthiopsis incrustans* isolate RRFMV14–5 after 7-day incubation at a temperature range of 18 to 38 C.

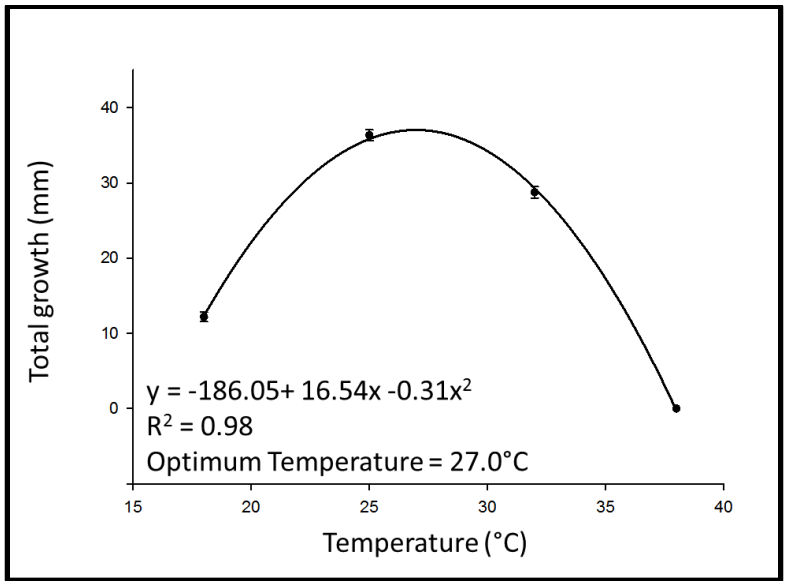


Figure B.124 Total growth of *Magnaporthiopsis cynodontis* isolate RS3–1 after 7-day incubation at a temperature range of 18 to 38 C.

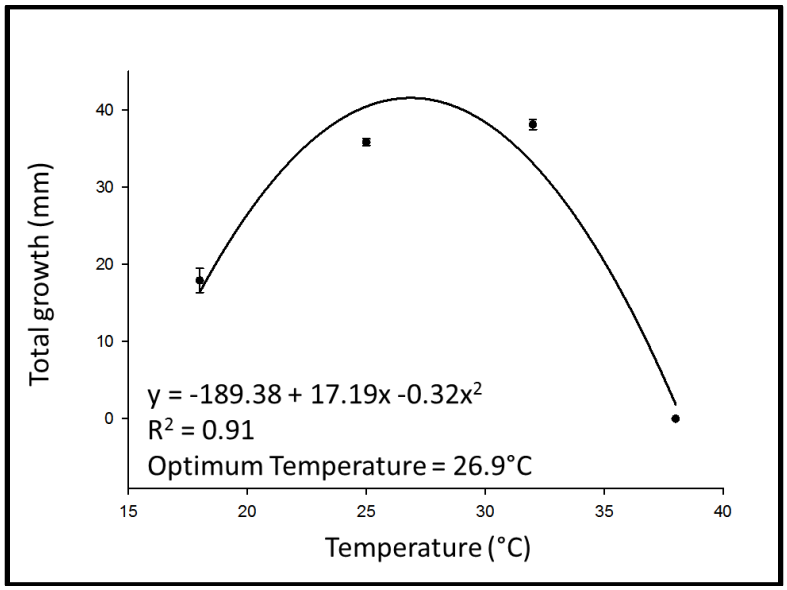


Figure B.125 Total growth of *Magnaporthiopsis cynodontis* isolate RS5–5 after 7-day incubation at a temperature range of 18 to 38 C.

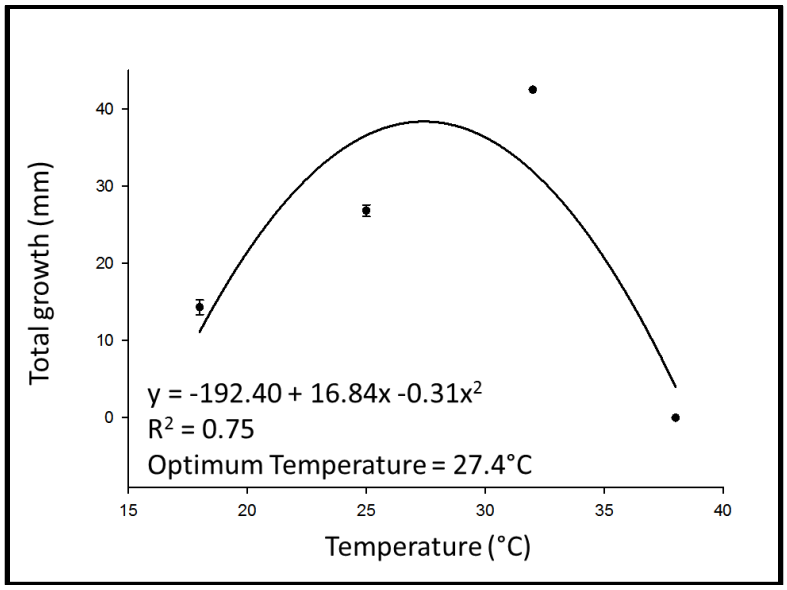


Figure B.126 Total growth of *Gaeumannomyces paulograminis* isolate RS7–1 after 7-day incubation at a temperature range of 18 to 38 C.

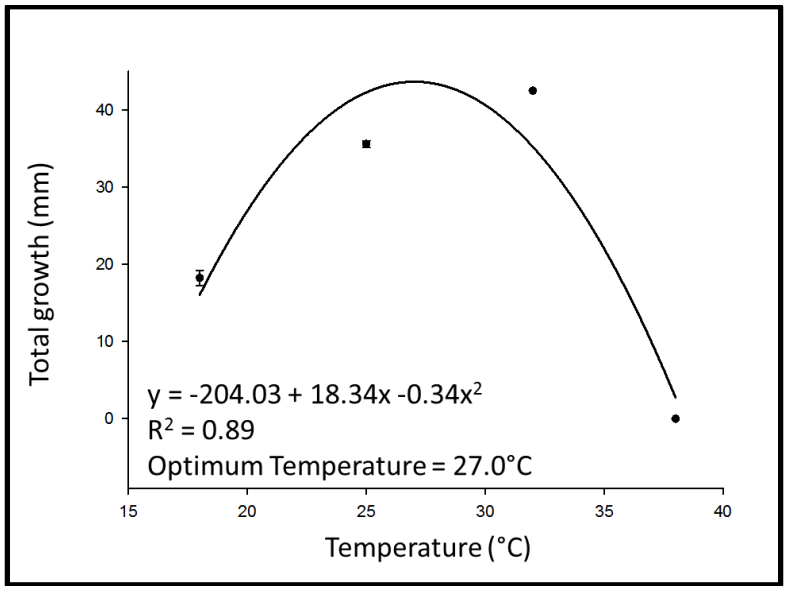


Figure B.127 Total growth of *Magnaporthiopsis cynodontis* isolate RS7–2 after 7-day incubation at a temperature range of 18 to 38 C.

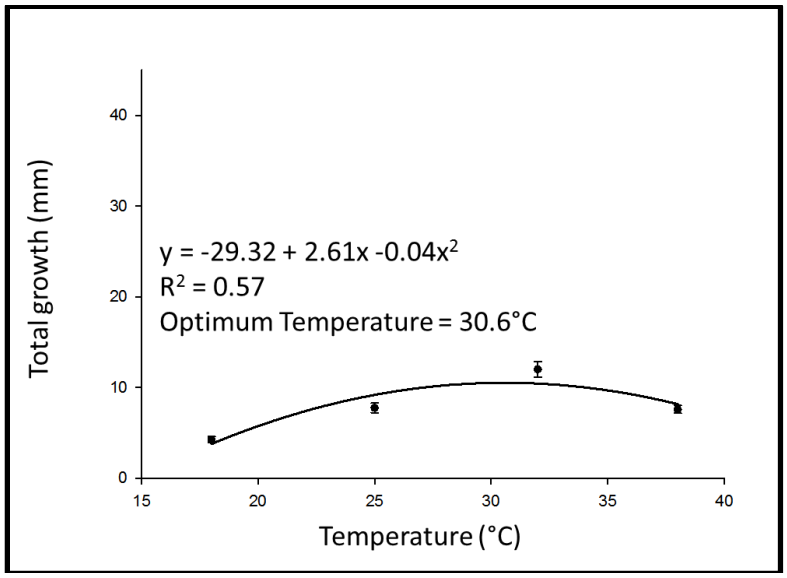


Figure B.128 Total growth of *Pseudophialophora cynodontis* isolate RW3–4 after 7-day incubation at a temperature range of 18 to 38 C.

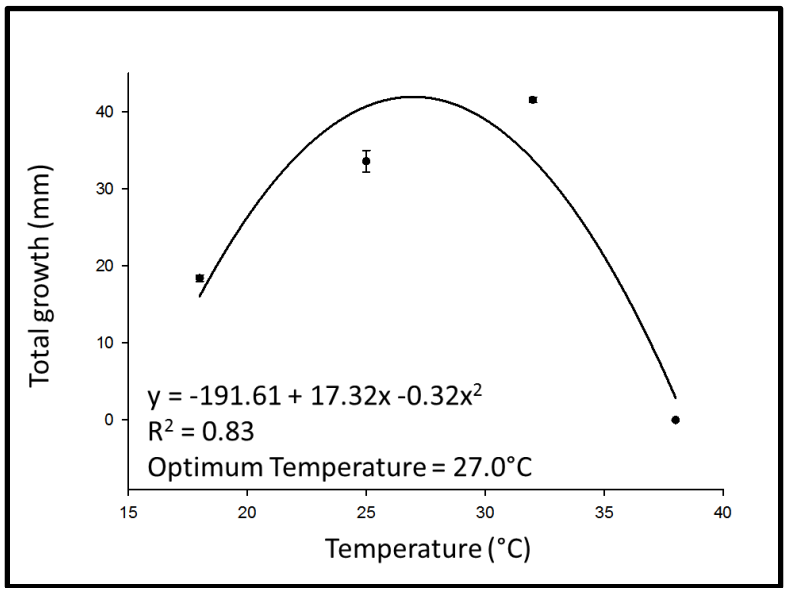


Figure B.129 Total growth of *Magnaporthiopsis poae* isolate SPKBG7 after 7-day incubation at a temperature range of 18 to 38 C.

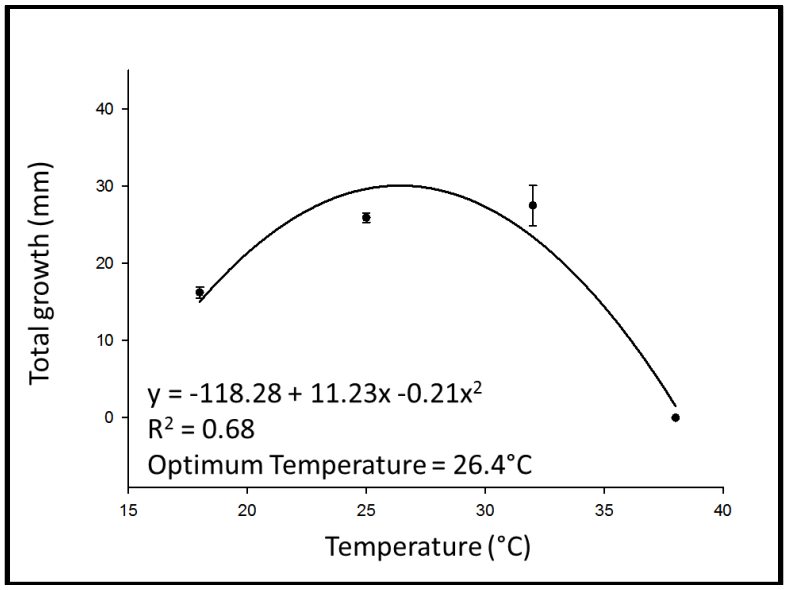


Figure B.130 Total growth of *Magnaporthiopsis poae* isolate TAP35 after 7-day incubation at a temperature range of 18 to 38 C.

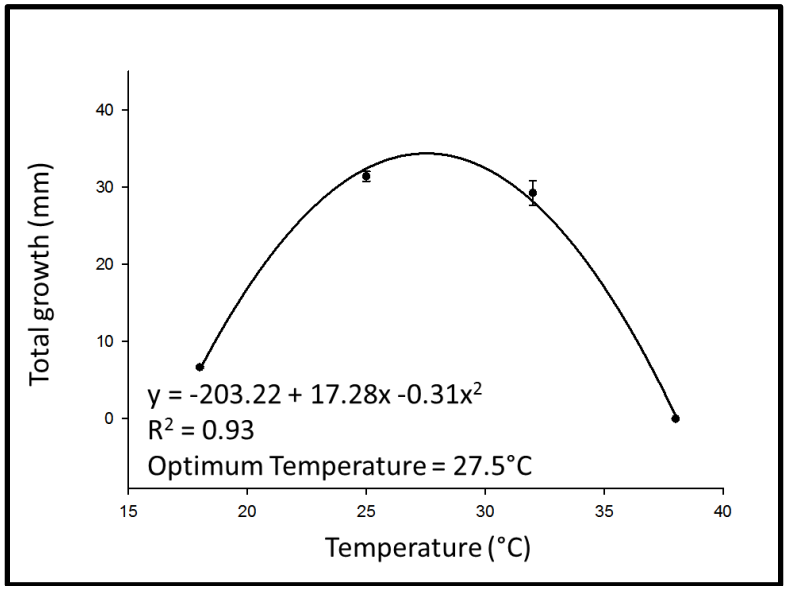


Figure B.131 Total growth of *Magnaporthiopsis poae* isolate TAP41 after 7-day incubation at a temperature range of 18 to 38 C.

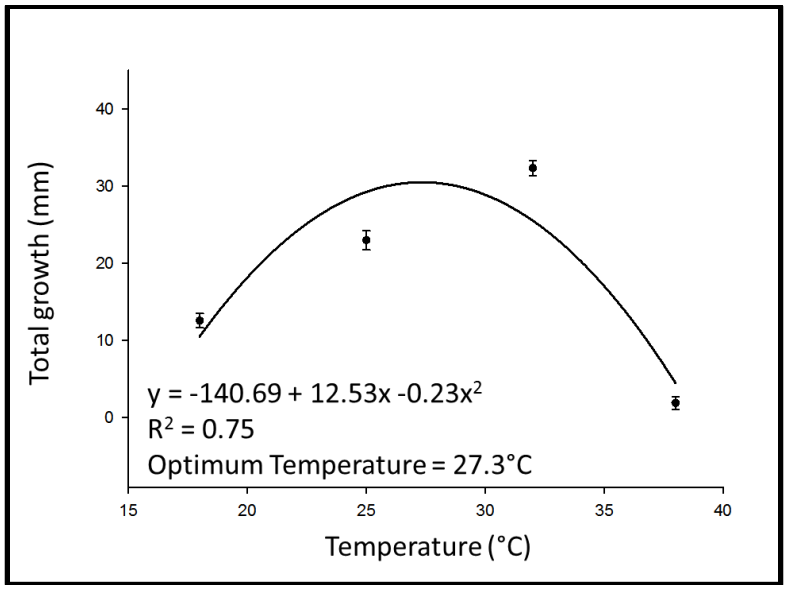


Figure B.132 Total growth of *Magnaporthiopsis taurocanis* isolate TPC4–5 after 7-day incubation at a temperature range of 18 to 38 C.

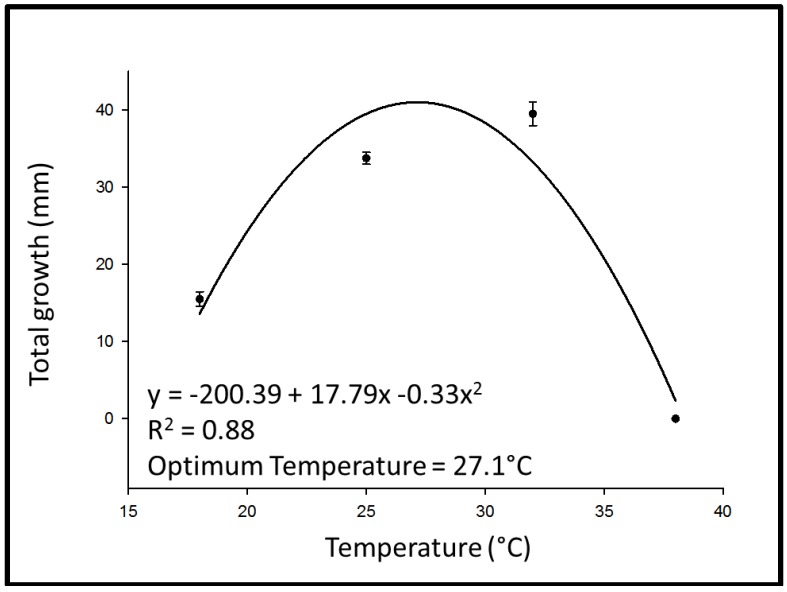


Figure B.133 Total growth of *Magnaporthiopsis taurocanis* isolate TPC5–3 after 7-day incubation at a temperature range of 18 to 38 C.

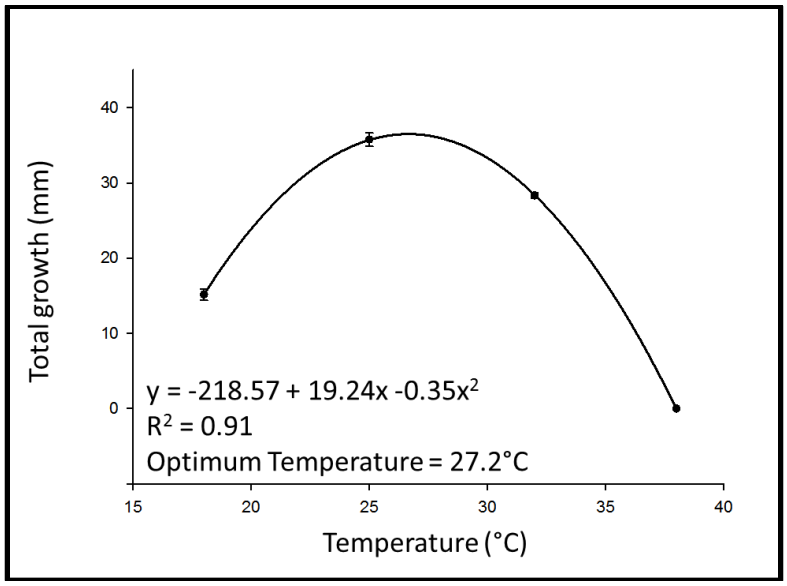


Figure B.134 Total growth of *Magnaporthiopsis incrustans* isolate WW3–5 after 7-day incubation at a temperature range of 18 to 38 C.

Figure B.135 Dried cultures of type specimens for storage at the U.S. National Fungus Collections Herbarium.

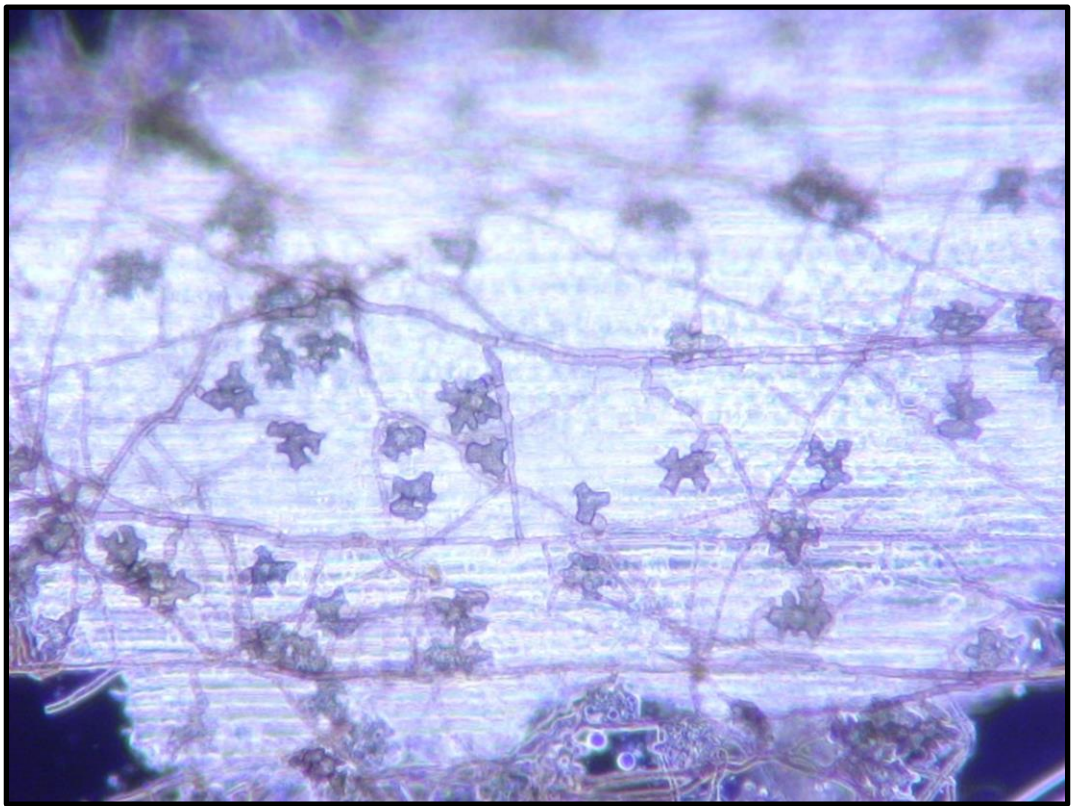


Figure B.136 Light micrograph showing hyphopodia of *Gaeumannomyces graminis* var. *graminis* at X400.

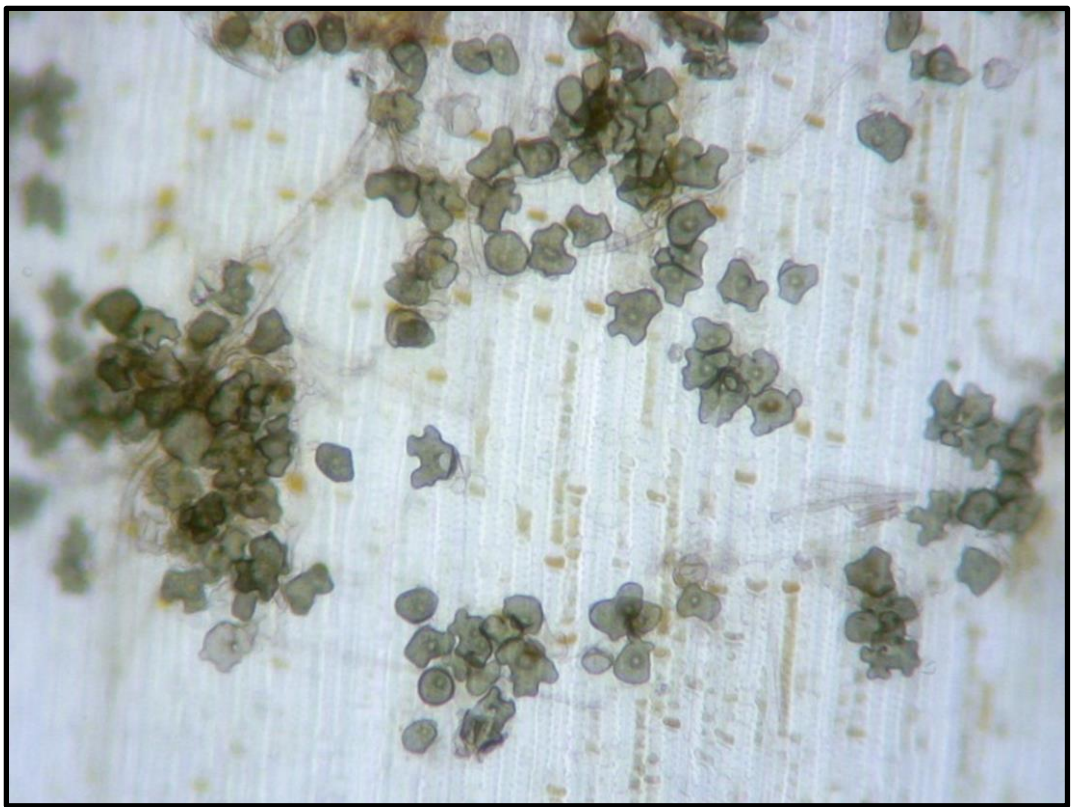


Figure B.137 Light micrograph showing hyphopodia of *Gaeumannomyces paulograminis* at X400.

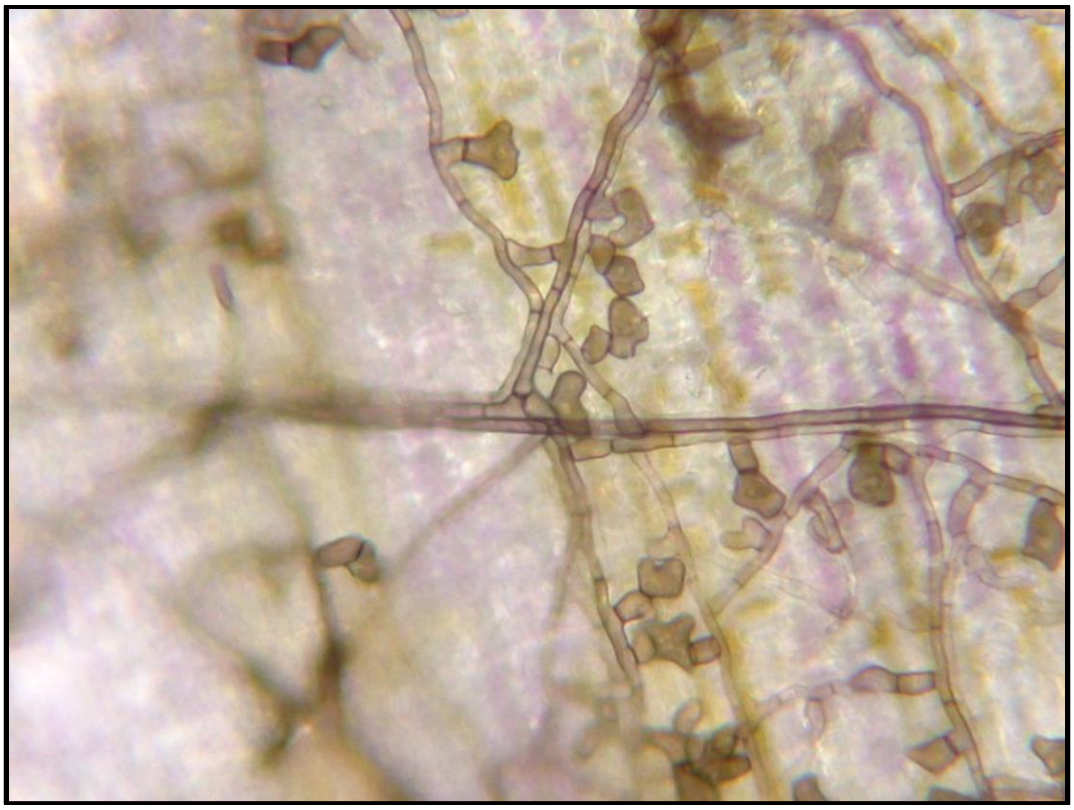


Figure B.138 Light micrograph showing hyphopodia of *Candidacolonium cynodontis* at X600.

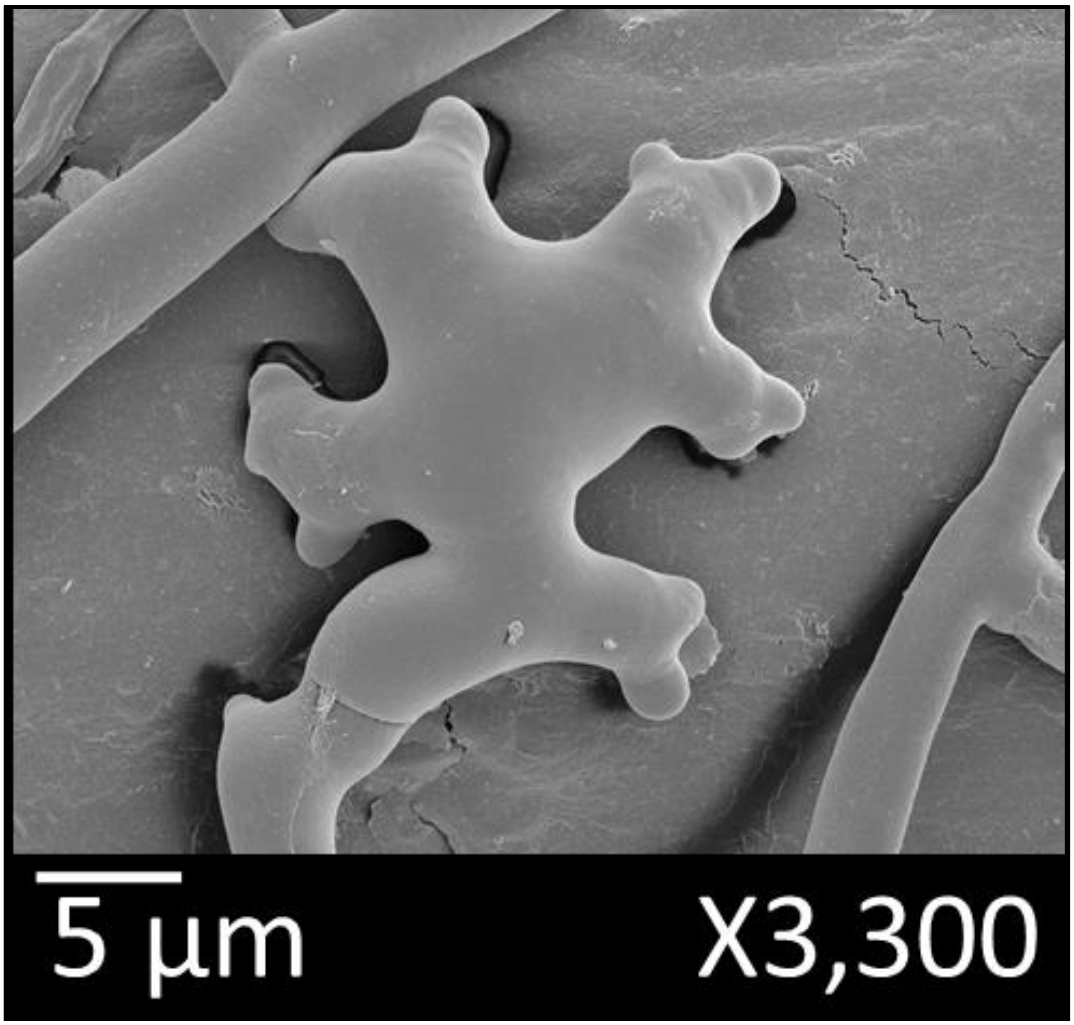


Figure B.139 Scanning electron micrograph showing hyphopodia of *Gaeumannomyces graminis* var. *graminis* at X3,300.

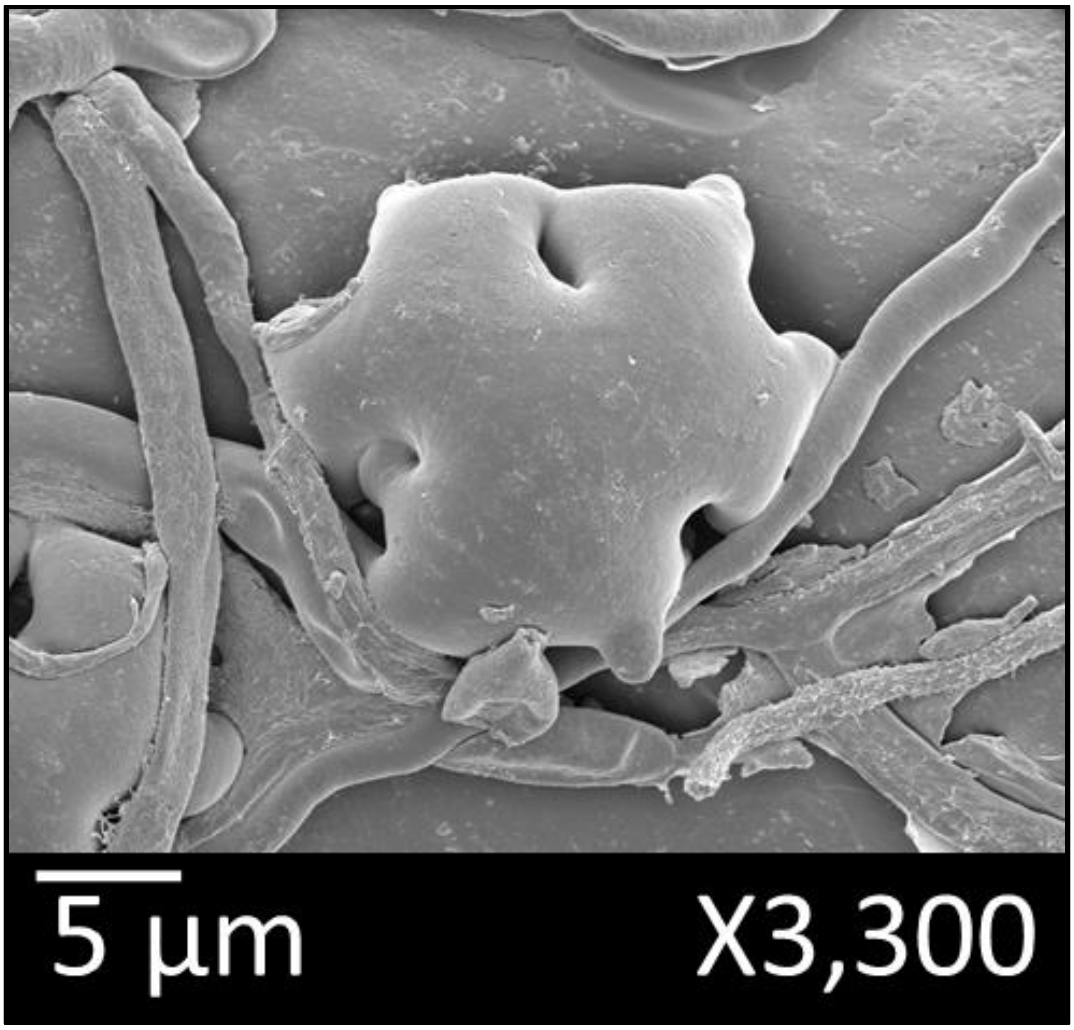


Figure B.140 Scanning electron micrograph showing hyphopodia of *Gaeumannomyces paulograminis* at X3,300.

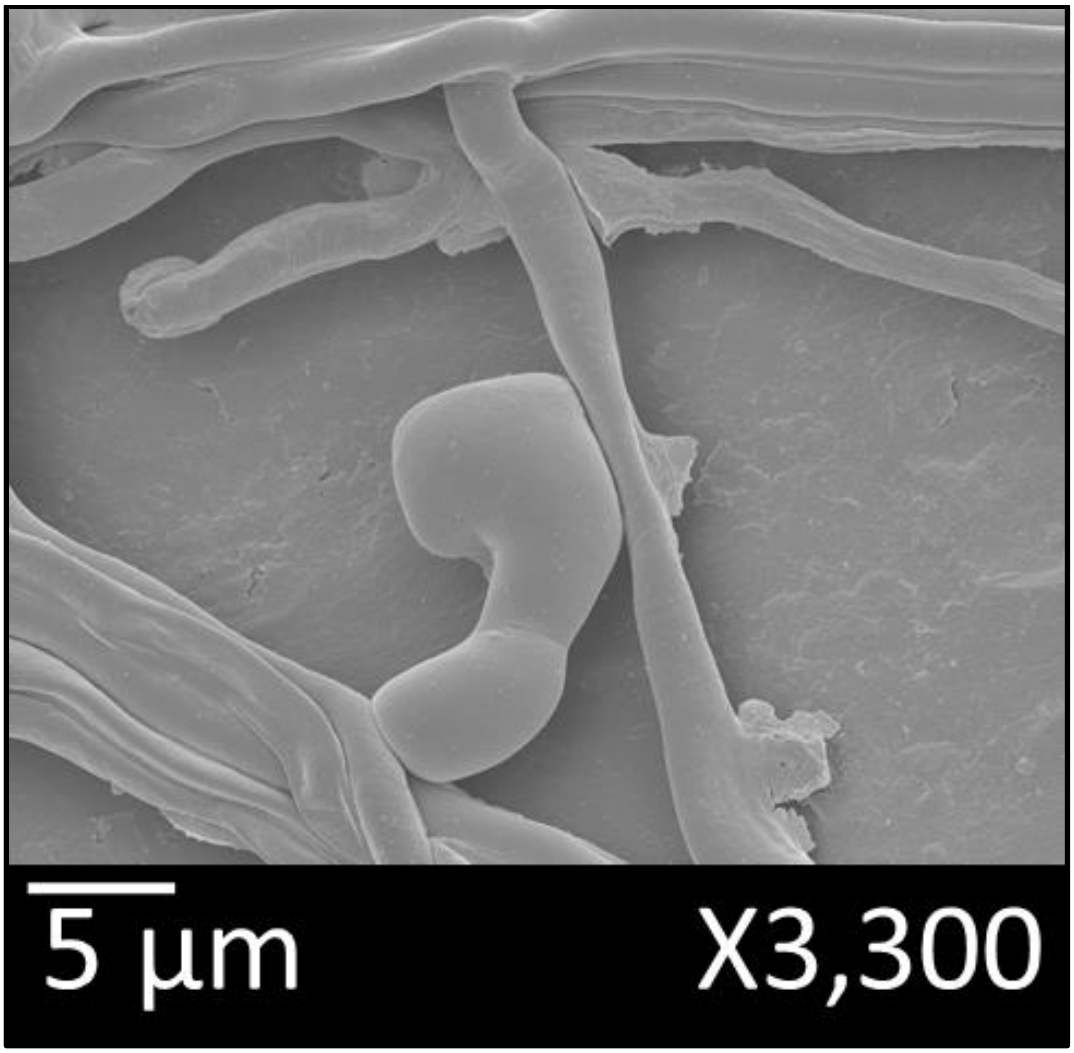


Figure B.141 Scanning electron micrograph showing hyphopodia of *Candidacolonium cynodontis* at X3,300.

APPENDIX C

SUPPLEMENTARY MATERIAL FOR PATHOGENICTY EVALUATIONS

Figure C.1 10.8 cm diameter samples of ultradwarf bermudagrass collected from research plots at the Rodney R. Foil research center

Figure C.2 Stoloniferous plant material extending from perimeter of turf sample.

Figure C.3 Collection of plant material consisting of five nodes and four internodes for pathogenicity evaluations.

Figure C.4 Soil autoclave at Rodney R. Foil Research Center

Figure C.5 Sterilized sand filled to within 2 cm of the top of inoculation containers (7.6 cm diameter) for pathogenicity evaluations.

Figure C.6 One square centimeter sections of PDA, fully colonized with fungal material, placed directly on top of sterilized sand for pathogenicity evaluations.

Figure C.7 Plant material, 25 nodes total, placed directly in contact with inoculum source for pathogenicity evaluations.

Figure C.8 Sterilized sand placed on top of plant material to retain moisture for pathogenicity evaluations.

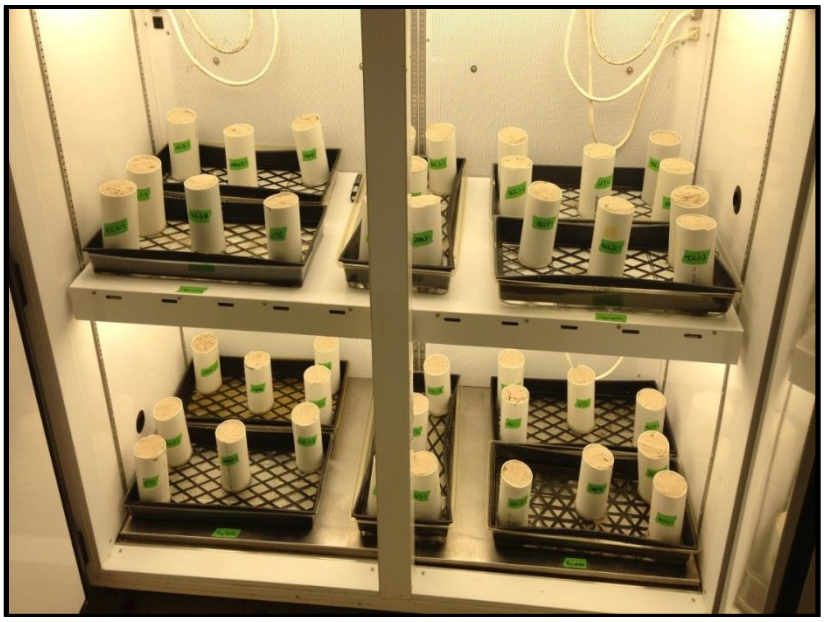


Figure C.9 Placement of inoculation containers in growth chamber for pathogenicity evaluations.

Figure C.10 WatchDog B-Series Button Logger used to record soil temperature of inoculation containers used for pathogenicity evaluations.

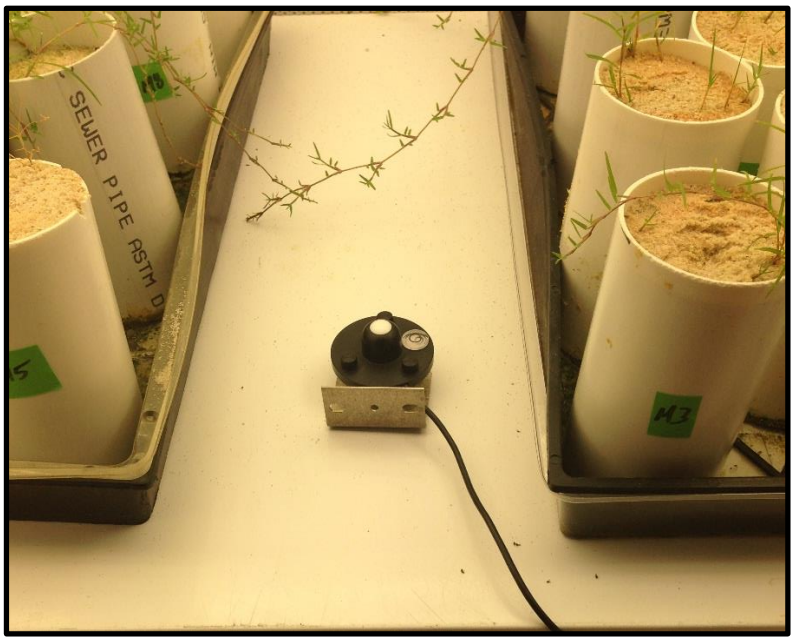


Figure C.11 Lightscout Quantum Sensor used to record photosynthetically active radiation in growth chambers used for pathogenicity evaluations.

Figure C.12 EL-USB-2-LCD data logger used to measure air temperature, relative humidity, and dew point in growth chambers used for pathogenicity evaluations.

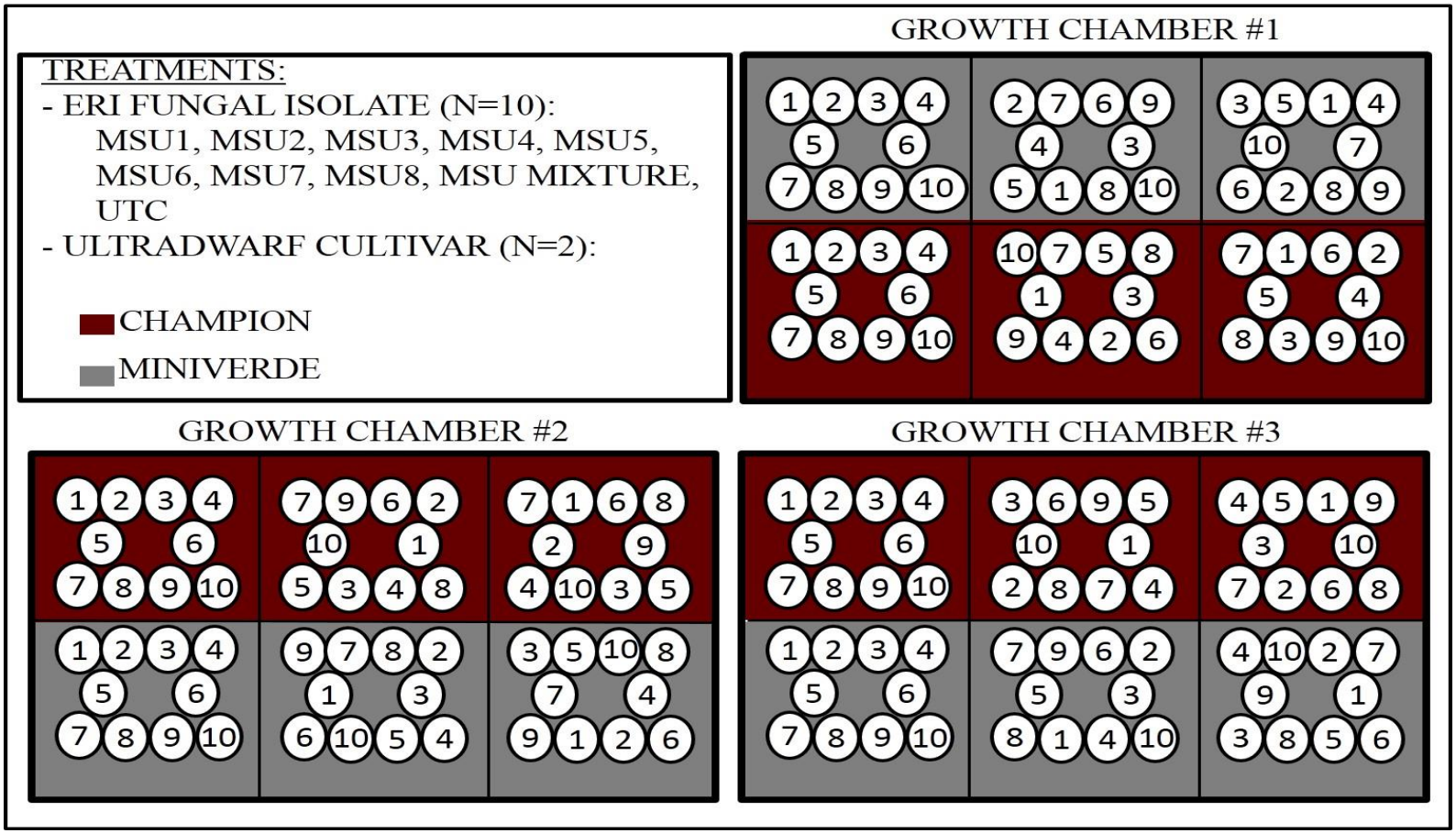


Figure C.13 Schematic illustration of experimental design and layout of pathogenicity evaluation.

MSU1 = *Gaeumannomyces graminis* var. *graminis*, MSU2 = *G. paulograminis*, MSU3 = *Magnaporthiopsis incrustans*, MSU4 = *M. hawaiiensis*, MSU5 = *M. taurocanis*, MSU6 = *M. cynodontis*, MSU7 = *Candidacolonium cynodontis*, MSU8 = *Pse*udophialophora *cynodontis*, MSU9 = Composite of MSU1 – MSU8, MSU10 = Untreated Control (UTC).

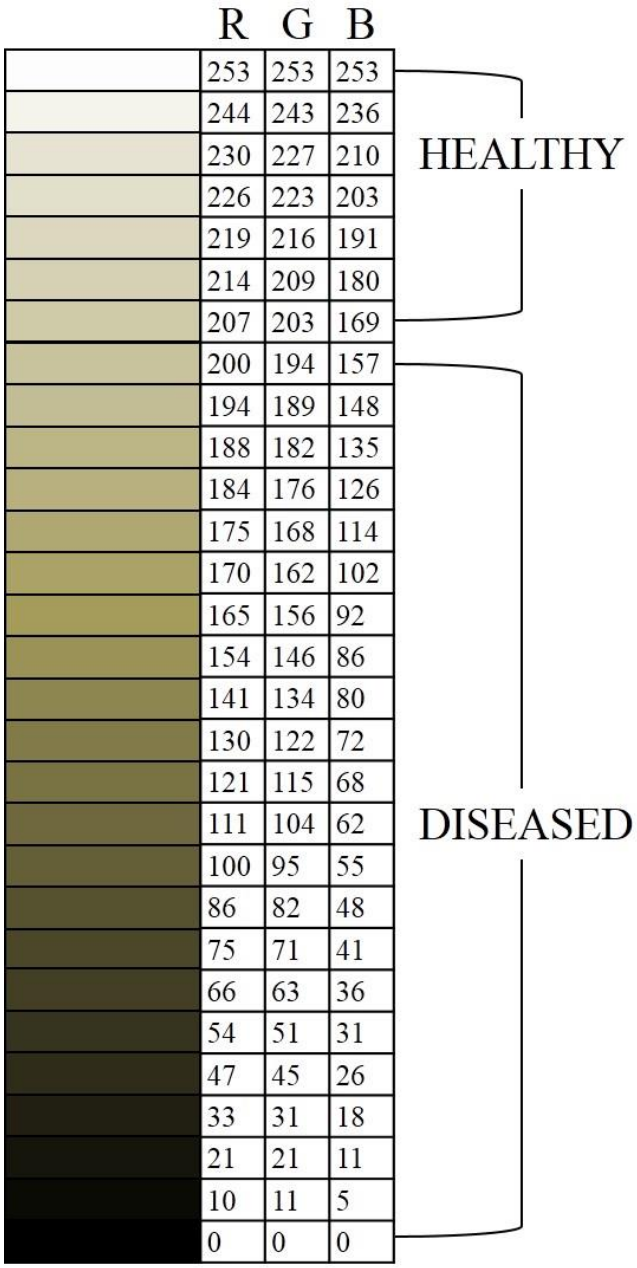


Figure C.14 Color classes and groups used for percent disease assessment via pixel color analysis in WinRhizo.

Corresponding red, green, and blue (R, G, and B, respectively) values are presented alongside respective colors.

Table C.1 ANOVA for total number of nodes (TNN) of ultradwarf bermudagrass samples from ectotrophic root-infecting fungi pathogenicity experiments conducted in Starkville, MS from August to October, 2014.

Source	DF	F-value	Pr > F
Experiment	2	4.15	0.1058
Error 1	———	———	———
Cultivar (Cv)	1	1.09	0.3261
Error 2	———	———	———
Fungal Species (FS)	9	2.60	0.0083
Cv × FS	9	0.63	0.7722
Error 3	———	———	———

Table C.2 ANOVA for total number of root-producing nodes (TNRPN) of ultradwarf bermudagrass samples from ectotrophic root-infecting fungi pathogenicity experiments conducted in Starkville, MS from August to October, 2014.

Source	DF	F-value	Pr > F
Experiment	2	9.52	0.0301
Error 1	———	———	———
Cultivar (Cv)	1	19.55	0.0022
Error 2	———	———	———
Fungal Species (FS)	9	2.81	0.0046
Cv × FS	9	0.67	0.7316
Error 3	———	———	———

Table C.3 ANOVA for frequency of fungal occurrence (FFO) of ultradwarf bermudagrass samples from ectotrophic root-infecting fungi pathogenicity experiments conducted in Starkville, MS from August to October, 2014.

Source	DF	F-value	Pr > F
Experiment	2	1.00	0.4444
Error 1	———	———	———
Cultivar (Cv)	1	1.00	0.3466
Error 2	———	———	———
Fungal Species (FS)	9	545.00	< 0.0001
Cv × FS	9	1.00	0.4429
Error 3	———	———	———

Table C.4 ANOVA for total root length (TRL) of ultradwarf bermudagrass samples from ectotrophic root-infecting fungi pathogenicity experiments conducted in Starkville, MS from August to October, 2014.

Source	DF	F-value	Pr > F
Experiment	2	7.86	0.0412
Error 1	———	———	———
Cultivar (Cv)	1	9.74	0.0142
Error 2	———	———	———
Fungal Species (FS)	9	2.01	0.0418
Cv × FS	9	0.44	0.9141
Error 3	———	———	———

Table C.5 ANOVA for percent disease (PD) of ultradwarf bermudagrass samples from ectotrophic root-infecting fungi pathogenicity experiments conducted in Starkville, MS from August to October, 2014.

Source	DF	F-value	Pr > F
Experiment	2	0.44	0.6730
Error 1	———	———	———
Cultivar (Cv)	1	0.12	0.7350
Error 2	———	———	———
Fungal Species (FS)	9	13.19	< 0.0001
Cv × FS	9	0.84	0.5823
Error 3	———	———	———

Table C.6 ANOVA for gravimetric root weight (GRW) of ultradwarf bermudagrass samples from ectotrophic root-infecting fungi pathogenicity experiments conducted in Starkville, MS from August to October, 2014.

Source	DF	F-value	Pr > F
Experiment	2	21.24	0.0074
Error 1	———	———	———
Cultivar (Cv)	1	23.61	0.0013
Error 2	———	———	———
Fungal Species (FS)	9	2.32	0.0181
Cv × FS	9	1.02	0.4307
Error 3	———	———	———

Table C.7 ANOVA for gravimetric stolon, rhizome, and foliage weight (GSRFW) of ultradwarf bermudagrass samples from ectotrophic root-infecting fungi pathogenicity experiments conducted in Starkville, MS from August to October, 2014.

Source	DF	F-value	Pr > F
Experiment	2	12.71	0.0185
Error 1	———	———	———
Cultivar (Cv)	1	2.92	0.1260
Error 2	———	———	———
Fungal Species (FS)	9	2.77	0.0051
Cv × FS	9	1.33	0.2240
Error 3	———	———	———

Figure C.15 Healthy ultradwarf bermudagrass sample after 8-week inoculation with untreated control treatment

Figure C.16 Ultradwarf bermudagrass sample after 8-week inoculation with *Gaeumannomyces graminis* var. *graminis*.

Figure C.17 Ultradwarf bermudagrass sample after 8-week inoculation with *Gaeumannomyces paulograminis*.

Figure C.18 Ultradwarf bermudagrass sample after 8-week inoculation with *Magnaporthiopsis incrustans*.

Figure C.19 Ultradwarf bermudagrass sample after 8-week inoculation with *Magnaporthiopsis hawaiiensis*.

Figure C.20 Ultradwarf bermudagrass sample after 8-week inoculation with *Magnaporthiopsis cynodontis*.

Figure C.21 Ultradwarf bermudagrass sample after 8-week inoculation with *Magnaporthiopsis taurocanis*.

Figure C.22 Ultradwarf bermudagrass sample after 8-week inoculation with *Candidacolonium cynodontis*.

Figure C.23 Ultradwarf bermudagrass sample after 8-week inoculation with *Pseudophialophora cynodontis*.

Figure C.24 Ultradwarf bermudagrass sample after 8-week inoculation with composite treatment.

Figure C.25 Stolons of ultradwarf bermudagrass samples after 8-week inoculation with *Gaeumannomyces graminis* var. *graminis*.

Figure C.26 Stolons of ultradwarf bermudagrass samples after 8-week inoculation with *Gaeumannomyces paulograminis*.

Figure C.27 Stolons of ultradwarf bermudagrass samples after 8-week inoculation with *Candidacolonium cynodontis*.

APPENDIX D

ENVIRONMENTAL DATA

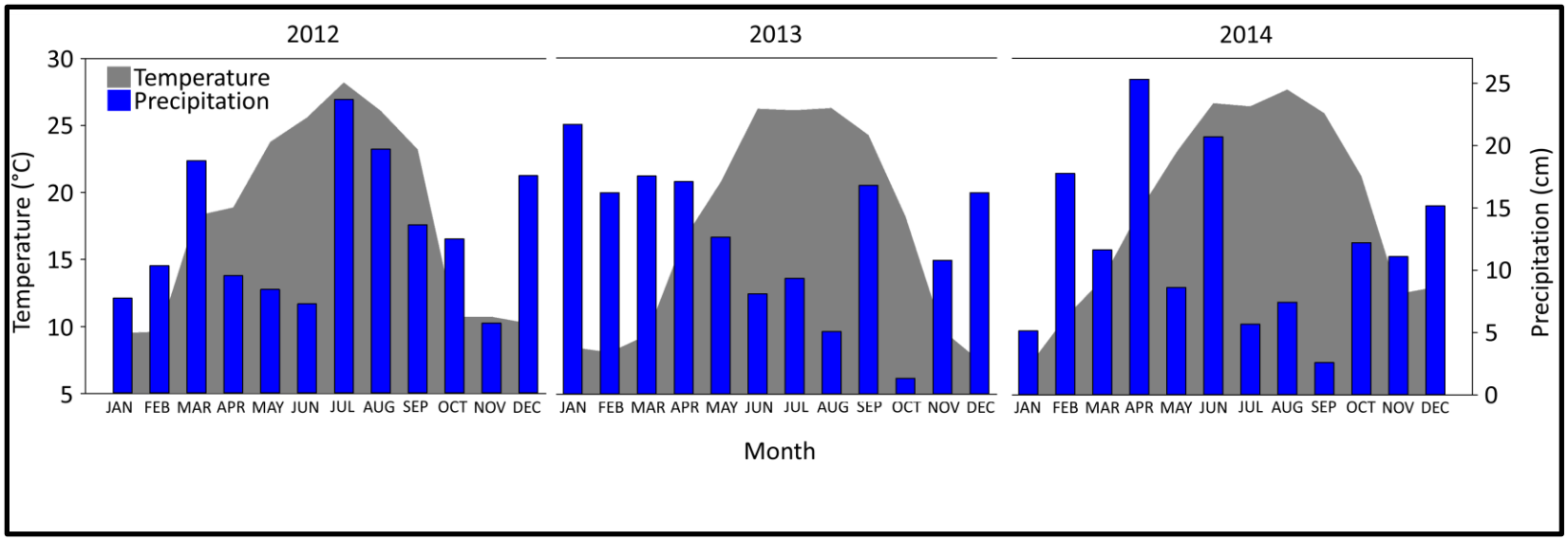


Figure D.1 Temperature and precipitation data for Starkville, MS during 2012, 2013, and 2014.