ABSTRACT

YAN YAN, Characterization, Pathogenicity and Management of *Rhizoctonia* Species Associated with Leaf and Sheath Spot of Ultradwarf Bermudagrasses. (Under the director of James P. Kerns)

Rhizoctonia leaf and sheath spot, also known as “mini-ring” on bermudagrass, is caused by the fungi *Rhizoctonia zeae* and *R. oryzae*. It is one of three major diseases of turfgrass caused by members of the genus *Rhizoctonia*. The most commonly isolated pathogen from bermudagrass with disease symptoms is *R. zeae*. Twenty isolates of a fungus resembling *R. zeae* were obtained from bermudagrass putting greens exhibiting ring symptoms in 2013 and 2014. Characterization by morphological techniques and polymerase chain reaction (PCR) showed that 17 isolates were tentatively identified as *R. zeae* and 3 were tentatively identified as *W. circinata var. zeae* when compared with sequences in the GenBank database. Cluster analysis based on sequence data separated the isolates collected in 2013 and 2014 into different groups. Pathogenicity of the isolates was determined by inoculating the hybrid bermudagrass ‘Champion’. Inoculated plants were incubated in a growth chamber at 34/30 °C (day/night) under conditions of 100% humidity. After seven days, all isolates were pathogenic, with no difference observed among isolates in the level of disease. These results demonstrated that *R. zeae* is a pathogen of hybrid bermudagrass under hot and humid conditions.

Seven growth chambers, with temperature settings at 10, 15, 20, 25, 30, 35, and 40 °C, were used to evaluate the effects of temperature on the radial growth of seven isolates of *R. zeae* collected in 2013. The optimum temperature for growth was 30°C across the seven isolates.
The sensitivity of seven isolates collected in 2013 was tested with 16 fungicides (flutolanil, pyraclostrobin, fluoxastrobin, azoxystrobin, propiconazole, triticonazole, triadimefon, pencyopyrad, fluxapyroxad, azoxystrobin+difenoconazole, tebuconazole, difenoconazole, iprodione, polyoxin-D zinc salt, fluxapyroxad+pyraclostrobin, and chlorothalonil), and the sensitivity of 13 isolates obtained in 2014 was tested with 5 different fungicides (chlorothalonil, azoxystrobin, pyraclostrobin, difenoconazole, and flutolanil). In general, all of the isolates were extremely sensitive to DMI and SDHI fungicides (EC$_{50}$ < 1 mg a.i. L$^{-1}$). Tebuconazole was the most effective fungicide in suppressing hyphal growth, iprodione and chlorothalonil were moderately effective, and all isolates showed insensitivity to QoI fungicides (EC$_{50}$ > 10 mg a.i. L$^{-1}$).

The efficacy of 9 fungicides (propiconazole, triticonazole 30.1%, triticonazole 19.2%, tebuconazole, azoxystrobin + difenoconazole, trifloxystrobin + triadimefon, pyraclostrobin + fluxapyroxad, polyoxin-D zinc salt, iprodione + trifloxystrobin) in controlling *Rhizoctonia* leaf and sheath spot was evaluated twice in 2014 and in 2015 on two different golf greens in NC. In 2014, disease symptoms were first observed in early July on one golf green, only applications of tebuconazole, 30.1% triticonazole, and 19.2% triticonazole reduced disease severity compared to the untreated control. In 2015, turfgrass quality differences were observed after the first application of fungicides, plots treated with fungicides had better turf quality than control plots. After a second application, a decrease in turf quality in plots treated with DMI fungicides was detected compared to the untreated control plots. Those treated with non-DMI fungicides showed better turf quality than the untreated control, but none were statistically different.
Characterization, pathogenicity and management of *Rhizoctonia* species associated with leaf and sheath spot of ultradwarf bermudagrass

by
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A thesis submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the degree of Master of Science

Plant Pathology

Raleigh, North Carolina
2016

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DEDICATION

I wish to dedicate this document to my parents. Thank you for your love and support.
BIOGRAPHY

Yan Yan was born on Feb. 15, 1990 in Beijing, China. She completed her Bachelor of Science degree in Turfgrass Management from Beijing Forestry University and Michigan State University in 2013. As an undergraduate student, Yan spent time as an intern in Jim Kerns’ lab as an undergraduate research assistant. Working in a plant pathology lab inspired her to continue her studies and in the fall of 2013, Yan accepted a graduate research program at North Carolina State University in the Department of Plant Pathology to pursue her Master of Science degree.
ACKNOWLEDGMENTS

I would like to thank my committee members, Dr. James P. Kerns, H. David Shew, and Marc A. Cubeta. All have given me excellent guidance, advice, and support through the duration of my program. I would also like to thank Lee Butler, Mike Soika, Jill Ploetz, and Ben Van Ryzin, for their support, and friendship, they helped me with collection and fungicide application. Thanks also to the Center for Turfgrass and Environmental Research at North Carolina State University for the financial support of this project.

I would like to thank my parents for their support and guidance during my entire college career.
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Chapter 1. Literature review

Introduction to *Rhizoctonia* genus

*Rhizoctonia* species are primarily soilborne fungi that cause disease in a wide range of plants. The most important *Rhizoctonia* species associated with plant disease formerly belonged to three major groups of the Basidiomycota: 1) the *R. solani* Kühn complex, multinucleate species with three or more nuclei per cell and a *Thanatephorus* Donk teleomorph; 2) binucleate *Rhizoctonia* spp. with a *Ceratobasidium* Rogers teleomorph; 3) and the *R. zeae* and *R. oryzae* groups with multinucleate species and a *Waitea* Warcup & Talbot teleomorph (Ogoshi 1987, Vilgalys and Cubeta 1994). The 2007 AFTOL (Assembling the Fungal Tree Of Life) classification of fungi included 17 orders of Agaricomycetes, three of which contained species formerly classified as Heterobasidiomycetes: the *Auriculariales*, *Sebacinales*, and *Cantharellales*. The *Cantharellales* contains *R. solani* and *R. zeae* (Hibbett, Bauer et al. 2014). Important characteristics of the *Rhizoctonia* genus are the lack of asexual spores (conidia), clamp connections, and rhizomorphs (Burpee and Martin 1992) that typify other members of the Basidiomycota. Ogoshi (1987) provided a detailed description of the characteristics specific to *R. solani*: teleomorph is *Thanatephorus cucumeris*, hyphae are multinucleate, hyphae and sclerotia are typical of *Rhizoctonia*, and the diameter of vegetative hyphae is 8-12 µm. The anamorphic classification of *Rhizoctonia* spp. is first based on the number of nuclei per hyphal cell, multinucleate (MNR), binucleate (BNR), and uninucleate (UNR). Based on the hyphal anastomosis reactions between isolates, multinucleate *R. solani* (teleomorph: *Thanatephorus cucumeris*) can be divided into 13 Anastomosis Groups (AGs) (AG1-13) (Sneh, Burpee et al. 1991, Carling, Kuninaga et al. 2002), with many groups
containing multiple subgroups. Currently, the ribosomal DNA internal transcribed spacer (ITS) is the barcode marker that is an appropriate region of the genome for classifying species of *Rhizoctonia* (González, D., 2013, Sharon, Sneh et al. 2006).

**Rhizoctonia disease on turfgrass**

Of all turfgrass diseases, *Rhizoctonia* diseases are likely the most common in tropical and subtropical climates and can cause severe damage to both cool- and warm-season grasses (Kerns and Tredway 2013). *Rhizoctonia* is a very diverse fungal genus that contains more than 100 species (Ogoshi 1987). Isolates of the multinucleate species *R. solani*, *R. oryzae* Ryker & Gooch, *R. zeae* Voorhees, and *Waitea circinata var. circinata* have been collected from turfgrasses (Burpee and Martin 1992). *R. solani* AG 2-IIIB is the causal agent of brown patch of cool-season grass, AG 2-2 LP causes large patch on warm-season grass. *R. oryzae* and *R. zeae* are the causal agents of leaf and sheath spot and their teleomorph *Waitea circinata* causes brown ring patch of cool-season grass. *R. solani* and *W. circinata var. circinata* form buff to brown mycelium on potato dextrose agar (PDA), young colonies may be white, while *R. zeae* and *R. oryzae* form white to buff to salmon-colored mycelium on PDA. *R. zeae* and *W. circinata* are closely related, (telemorph of *R. zeae* is *Waitea circinata var. zeae*), but are differentiated by the morphology and appearance of sclerotia. Sclerotia of *R. zeae* are uniform, spherical, and begin as white and become orange to salmon-colored. In contrast, the colors of sclerotia of *W. circinata var. circinata* are buff to dark brown and can be irregular in size and shape (Burpee and Martin 1992, Kammerer and Harmon 2008). Although most *Rhizoctonia* diseases in turfgrasses develop during warm/hot, humid conditions, yellow patch, caused by *R. cerealis*, occurs during cool, wet periods and is mostly associated with cool-season turfgrasses. *R. cerealis* (=*Ceratobasidium cereale* D.I. Murray
& Burpee) is characterized by having two nuclei per cell and grows best when temperatures are between 14 and 20 °C. (Martin and Lucas 1983).

**Introduction to Rhizoctonia leaf and sheath spot**

Martin et al. (1983) were the first to associate *R. zeae* with a disease in turfgrass in the U.S. Brown patch caused by *R. zeae* can severely blight tall fescue under high temperatures (>32 °C), but under cooler temperatures, *R. zeae* is less aggressive than isolates of *R. solani* (Martin and Lucas 1984) on turfgrasses. Since this first report, *R. zeae* has been reported from many hosts worldwide. On corn, *R. zeae* is less aggressive than *R. solani* AG 2 and AG 4, which causes severe necrosis on hypocotyls, but it is more aggressive at high temperatures (34/20°C day/night) (Sumner and Bell 1982). *R. zeae* isolates from onion had optimum growth at 32 °C compared to *R. solani*, which had optimum growth at 25-30 °C (Erper, Karaca et al. 2006, Tomaso-Peterson and Trevathan 2007). Isolates of *R. zeae* were reported affecting *Panicum tennesseense* Ashe in Rhode Island and also had an optimum temperature for growth of 32 °C (Mitkowski 2003). In 2002, *R. zeae* and *R. solani* were isolated from *Lolium perenne* and *Festuca* spp. with symptoms of brown patch in Hungary, the symptoms observed were necrotic lesions on the roots and stems, and brown lesions on leaves. *R. zeae* colonies were buff-colored, fast-growing, with small, ball-shaped, reddish-colored sclerotia 0.5-0.9 mm in diameter (Vajna and Oros 2005).

The teleomorph of *R. zeae*, *W. circinata var. circinata*, is pathogenic on cool-season grasses (Toda et al., 2005). They isolated an unidentified *Rhizoctonia* spp. from creeping bentgrass in Japan that exhibited tan to yellow-brown circular or irregular small patches ranging from 10 to 50 cm in diameter. The affected turf eventually developed brownish rings but the turf in the center of the rings recovered, and in some cases, it recovered completely.
The author discovered that the new isolates were multinucleate and compared it to known species morphologically and molecularly. They were most similar to *W. circinata var. circinata*, which is the teleomorph of *R. zeae*. Pathogenicity was tested demonstrating that it is virulent to creeping bentgrass, and named this disease brown ring patch (Toda, Mushika et al. 2005). *W. circinata var. circinata* was first confirmed to be a pathogen of annual bluegrass in the U.S. in 2007 (de la Cerda, Douhan et al. 2007). Since then, *W. circinata var. circinata* has been identified in Arizona, California, Connecticut, Idaho, Illinois, Massachusetts, Minnesota, New York, New Jersey, Ohio, Oregon, Rhode Island, Virginia, and Wisconsin (Kerns and Tredway 2013). Since brown ring patch is a relatively new disease, very few fungicides are labeled for it, and application of nitrogen was shown to reduce disease severity. The plant growth regulator Primo Maxx (trinexapac-ethyl, Syngenta) had little effect on disease severity (Wong, Chen et al. 2009). Research demonstrated that fungicides such as polyoxin-D zinc salt, azoxystrobin, and flutolanil provided high levels of control of brown ring patch (McDonald et al. 2014).

*Rhizoctonia* leaf and sheath spot is also known as “mini-ring” on bermudagrass. In 1999, *R. zeae* was isolated as the predominant species from bermudagrass with symptoms of leaf and sheath spot disease (Elliott 1999). This disease is now considered to be caused by *R. zeae* and *R. oryzae*, and is the third major *Rhizoctonia* disease of turfgrass. Of the two fungi, *R. zeae* is more commonly observed as it is pathogenic on both cool and warm-season turfgrass (Kammerer and Harmon 2008).

The outbreak of leaf and sheath spot became more frequent widespread after conversion from creeping bentgrass to ultra dwarf bermudagrass on putting greens. Symptoms of the disease have been observed on ultradwarf bermudagrass putting greens in
North Carolina, South Carolina, Georgia, Mississippi, Alabama, Louisiana, and Texas (Inguagiato and Martin 2015).

**Symptoms of *Rhizoctonia* leaf and sheath spot**

Symptoms of *Rhizoctonia* diseases vary greatly among turfgrass species, depending on soil and environmental conditions and the type of grass affected. Symptoms in low-cut grasses, such as those on golf greens, are different from symptoms in higher-cut grasses, such as those on home lawns (Smiley, Dernoeden et al. 1992). The initial symptoms of *Rhizoctonia* leaf and sheath spot are a bronze ring spot ranging from 2 to 10 cm that can expand to a foot in diameter (Figure 1.1). *Rhizoctonia* leaf and sheath spot also may form a “smoke ring” symptom similar to that observed in brown patch. Since bermudagrass grows vigorously in summer heat, the symptoms are usually first noticed in August and September. Sometimes the symptoms may persist throughout the winter and into spring resulting in necrotic rings (Burpee and Martin 1992, Smiley, Dernoeden et al. 1992, Inguagiato and Martin 2015).

**Ultradwarf Bermudagrasses**

Bermudagrass is a warm-season grass characterized by the C₄ photosynthetic pathway. C₄ plants are usually found between 30° N and 30° S latitudes and need fewer molecules of water than C₃ grass when one CO₂ molecule is fixed via photosynthesis. Therefore, the C₄ metabolic pathway provides warm-season grasses with an advantage for performing in hot and dry climates (Hanna, Raymer et al. 2013).

Bermudagrasses can be used as a high-quality turf for golf, commercial, athletic fields, parks, and homelawns. It is widely used in North Carolina, South Carolina, Georgia, Florida, Texas, Tennessee, Alabama, Arizona, and Mississippi. New bermudagrass cultivars
have been developed to improve the putting surface quality on greens planted with bentgrasses, especially in the transition zone where bentgrasses are not well adapted. The legendary Dr. Glenn Burton released several cultivars that revolutionized golf around the world, the first result of Dr. Burton’s research was Tiflawn, but it was so coarsely textured that it could not be used for putting greens; the Tifgreen was officially released in 1956 as the hybrid of Tiflawn and a fine textured cultivar *Cynodon transvaalensis* (Inguagiato and Martin 2015). It was the solution to the warm-season grass putting green problem and became very common on many golf courses in the South. Nine years later in 1965, a new cultivar, Tifdwarf, believed to be a natural mutation from Tifgreen was officially released, having shorter leaves, stems and tolerance to low mowing height (Inguagiato and Martin 2015). Tifdwarf remained the standard for bermudagrass putting greens for many years until it was replaced by ultradwarf cultivars in 1990s. Ultradwarf cultivars including, ‘Champion’, ‘Mini-Verde’, and ‘Tifeagle’, can tolerate very close mowing heights of 0.23 cm. Due to the lower mowing heights that ultradwarfs can tolerate and the sterility of ultradwarfs, numerous southern golf courses have switched to ultradwarfs for greater green speeds and improved quality (Inguagiato and Martin 2015).

**Management of *Rhizoctonia zeae***

The rapid increase of *R. zeae* as a pathogen of turfgrasses is attributed to the widespread use of ultradwarf bermudagrasses since 2010 and modern cultural practices. Ultradwarf bermudagrass, including ‘Mini Verde, Champion, and TifEagle’, generally have a shallow root system, so the risk of drought damage is greater on ultradwarf putting greens (Inguagiato and Martin 2015) than on formerly used bermudagrasses. Grass growing on a sandy root zone with great leaching potential will be prone to drought, and diseases that are
active on leaves, such as leaf and sheath spot, can be more widespread and damaging when root zones are dry and nutrients are limited (Inguagiato and Martin 2015). The use of reduced annual nitrogen rates over the past 40 years from 29-34 kg N per square meter to as low as 7 kg N per square meter to meet golfers demands for fast green speed has impacted putting performance. The impact of low nitrogen fertility may be most pronounced on sandy soils with low nutrient holding capacity or soil root zones modified with a thick sand topdressing layer (Inguagiato and Martin 2015). The increased use of ultradwarf bermudagrass cultivars, reduced nitrogen fertility, nutrient deficiency due to use sand based greens, and a high demand for low mowing height for fast green speed are all thought to have contributed to the relative recent outbreak of *Rhizoctonia* leaf and sheath spot. Golf course superintendents did not have an effective management strategy for this disease. Some superintendents implemented an integrated pest management strategy by increasing mowing height and incorporating topdressing and rolling, a strategy learned from previous research on cool-season greens (Inguagiato and Martin 2015).

Little has been done on the sensitivity of *R. zeae* to fungicides. Martin et al. (1984) reported that *Rhizoctonia*-like fungi collected from turfgrass were sensitive to benomyl, with EC$_{50} < 10$mg/L, whereas isolates of *R. zeae* collected from tall fescue were resistant to benomyl, with EC$_{50} > 50$mg/L, but were sensitive to the fungicides carboxin and PCNB. The author concluded that the tolerance of *R. zeae* to benomyl could not be fully evaluated since *R. zeae* had only recently been shown to induce foliar blight on turfgrass. This was confirmed by Carling et al. (1990). *R. zeae* and *R. oryzae* isolates appeared generally more sensitive to hexaconazole (EC$_{50} < 0.01$mg/L) and less sensitive to iprodione (EC$_{50} > 10$mg/L) and benomyl (EC$_{50} > 10$mg/L) and displayed a similar range of sensitivity to PCNB (EC$_{50} =$
2.9mg/L) and prochloraz (EC$_{50}$ = 1.49mg/L). In the in vivo sensitivity test, benomyl, carboxin, PCNB, iprodione, chlorothalonil, and triadimefon were sprayed on tall fescue and then challenged with isolates of R. zeae in a greenhouse. Disease severity was greater on benomyl treated plants than on non-treated plants, which may be attributed to the reduction in antagonistic microflora. PCNB was ineffective against R. zeae isolates, which was contrary to the in vitro tests result. Carboxin, triadimefon, iprodione, and chlorothalonil were effective in preventing R. zeae infection (Martin, Campbell et al. 1984). In another study, R. zeae isolates from rice were very sensitive to benomyl. They were tested against 14 fungicides with different modes of action, and the rice isolates were very sensitive to flusilazole (EC$_{90}$ = 2mg/L), propiconazole (EC$_{90}$ = 2mg/L), fenpropimorph (EC$_{90}$ = 0.2mg/L), and benomyl (EC$_{90}$ = 3mg/L), but less sensitive to benodanil (EC$_{90}$ = 14mg/L), pencycuron (EC$_{90}$ = 60mg/L), and iprodione (EC$_{90}$ = 58mg/L). The high sensitivity of R. zeae to benomyl were contrary to the results of Martin et al. (1984), the author speculated that there were either physiological and genetic differences between R. zeae from rice and turfgrass or that benomyl-resistant isolates of R. zeae existed frequently in nature (Kataria, Hugelshofer et al. 1991).

**Differential mode of action of fungicides**

For golf greens and fairways, applying fungicides to control disease is a common and routine practice. Resistance of Rhizoctonia species to benomyl has been shown in many studies (Martin, Lucas et al. 1984, Carling, Helm et al. 1990, Kataria, Hugelshofer et al. 1991). Based on the results from Amaradasa’s (2014) study, none of the three fungicides tested (iprodione, pyraclostrobin and triticonazole) were able to control all Rhizoctonia groups infecting cool-season turfgrasses. Since there are only a few studies about R. zeae
sensitivity to fungicides, we selected 16 fungicides (Table 1.1) and evaluated their efficacy to *R. zeae*.

The 16 fungicides belonged to 6 chemical classes (QoI-Quinone outside inhibitors, DMI – DeMethylation Inhibitors, SDHI – Succinate DeHydrogenase inhibitors, Polyoxin-D, Chloronitrile, and Dicarboximide) and two of them contained multiple classes (QoI + DMI, QoI + SDHI).

QoI fungicides belong to the mode of action (MOA) group C subgroup C3 targeting mitochondrial respiration at complex III. Respiration is a metabolic process that produces energy for all other cell functions. QoI (Quinone outside inhibitors) fungicides also called strobilurin fungicides, and include azoxystrobin, fluoxastrobin, and pyraclostrobin. They work by disrupting respiration by interfering with electron transport at complex III (Latin 2011). QoI compounds are recognized for their broad spectrum of activity and ability to inhibit spore germination and mycelial growth and reduce spore production in sensitive fungal pathogens QoI fungicides (strobilurins) are derived from naturally occurring compounds originally isolated from the mushroom forming fungus *Strobilulus tenacellus* that grow a saprobe on decaying forest plants, (Latin 2011).

Among turfgrass pathogens, it has reported that *Pyricularia grisea* Sacc (gray leaf spot), *Colletotrichum cereal* Manns, *Alternaria* spp., and *Pythium aphanidermatum* Edson are resistant to QoI fungicides. These strains possess an altered target site in their mitochondrial DNA that prevents the fungicide from interrupting electron flow and energy production in the cytochrome system (Ma, Felts et al. 2003, Latin 2011).

DMI (DeMethylation Inhibitors) fungicides are classified to MOA group G targeting sterol biosynthesis. Ergosterol as the main sterol in cell membranes of true fungi and is
important for membrane integrity. The toxic agent blocks the action of demethylase enzymes in the pathway from acetyl coenzyme A to ergosterol in the cytoplasm, and leads to an ergosterol deficiency. As a result, membranes are not able to properly regulate the compounds that enter and exit the cell, thus leakage occurs and growth stops (Latin 2011).

Phytotoxicity can occur on some turfgrasses when DMI fungicides are applied during a period of high temperature (>32°C) (Miller, et al. 2012). The observed phytotoxicity is the effect of the plant growth regulating compounds in DMI fungicides binding to cytochrome P-450, thus inhibiting gibberellin and sterol biosynthesis in plants (Elliott 1995).

Similar to QoI fungicides, SDHI (Succinate DeHydrogenase Inhibitor) fungicides belong to MOA group C2 that inhibit fungal respiration. Unlike the QoI fungicides, SDHI fungicides specifically bind to the ubiquinone-binding site of the mitochondria complex II. Similar to the QoI fungicides, the consistent use of these site-specific SDHI fungicides can result in the selection of resistant fungal genotypes (Avenot and Michailides 2010).

Although the existence of mutants resistant to flutolanil has been known for several years in several organisms (Avenot and Michailides 2010), none of the SDHI fungicides has been associated with resistance in fungi that cause diseases of turfgrass. Flutolanil has been reliably effective against Rhizoctonia species for more than 30 years without failure due to the occurrence of fungicide-resistant strains of the pathogen (Latin 2011).

Polyoxin-D a member of MOA group H targets glucan (chitin) synthesis. Polyoxins inhibit the generation of chitin, an essential component of cell walls in most fungi. Chitin is also a structural component of arthropod exoskeletons, and polyoxins have no effects on plants or mammals (Latin 2011). Polyoxins are antibiotics produced by a bacterium (Streptomyces cacao var. asoensis), and there are several variants of polyoxins (polyoxin A-
Polyoxin D is the metabolically active compound that suppresses disease of turfgrass caused by species of *Colletotrichum* and *Rhizoctonia* species (Latin 2011).

Chlorothalonil is a chloronitrile fungicide in MOA group M, a multi-site functional group, so it has a low risk of resistance. Chlorothalonil, classified as a benzonitrile, is a very broad-spectrum contact fungicide. It is the most important fungicide available for controlling turf disease and one of the more widely utilized fungicides for managing crop disease. The most significant target of chlorothalonil is the sulfhydryl group of the compound glutathione, which flows freely through the cytoplasm and is an essential regulator of normal cell metabolism. It supports enzymes that are necessary for breaking down complex molecules into simple nutrients and energy in the cellular fluid (Latin 2011). Chlorothalonil readily reacts with glutathione, rendering it inactive, and without glutathione, enzyme function at several sites within the cell are irreversibly impaired and cell metabolism is disrupted, leading to cell death. Chlorothalonil is also reported to block energy production in the cell by reacting with the sulfur-containing compound acetyl coenzyme A (Latin 2011).

Dicarboximide, from MOA group E, targets signal transduction in the pathogen. It defeats the pathogens’ environmental sensory systems by deceiving pathogens into overproducing substances that increase osmotic pressure, leading to swelling and eventual bursting of hyphal tips, effectively suppressing fungal growth (Latin 2011). The mode of action of dicarboximide was confirmed by Danneberger’s (1982) research on the effect of iprodione on *Drechslera sorokiniana*, the causal agent of leaf spot of *Poa annua*. They discovered that iprodione allowed conidia of *D. sorokiniana* to germinate in culture but the germination process progresses the tip of the hyphae swell and burst effectively limiting the elongation of germ tube.
Many fungicides have been approved that effectively control species of *Rhizoctonia* that are pathogens of turfgrass (Martin, Lucas et al. 1984, Carling, Helm et al. 1990, Kataria, Hugelshofer et al. 1991, Elliott 1995, Wong, Chen et al. 2009). *R. zeae*, as the causal agent *Rhizoctonia* disease leaf and sheath spot on bermudagrass and cool-season grass should get more attention, but there is no research regarding the efficacy of these fungicides on *R. zeae* *in vitro* or *in vivo*. My objectives are to: 1) molecularly and morphologically identify *R. zeae* and evaluate pathogenicity of *R. zeae* on ‘Champion’ bermudagrass, and 2) determine the *in vitro* and *in vivo* sensitivity of *R. zeae* to various fungicides.
Literature Cited


Fig. 1.1 “Ring” symptoms of *Rhizoctonia* leaf and sheath spot
Table 1.1 Group, mode of action, phytomobility, movement throughout the plant and among cells, and FRAC code of fungicides used in this study.

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Group</th>
<th>MOA</th>
<th>Phytomobility</th>
<th>Movement throughout the plant</th>
<th>Movement among cells</th>
<th>FRAC$^z$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoxastrobin</td>
<td>QoI</td>
<td>C3</td>
<td>Acropetal penetrant</td>
<td>Xylem mobile</td>
<td>Apoplastic</td>
<td>11</td>
</tr>
<tr>
<td>Pyraclostrobin</td>
<td>QoI</td>
<td>C3</td>
<td>Local penetrant</td>
<td>Translaminar</td>
<td>Apoplastic</td>
<td>11</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>QoI</td>
<td>C3</td>
<td>Acropetal penetrant</td>
<td>Xylem mobile</td>
<td>Apoplastic</td>
<td>11</td>
</tr>
<tr>
<td>Difenoconazole</td>
<td>DMI</td>
<td>G</td>
<td>Acropetal penetrant</td>
<td>Translaminar</td>
<td>Apoplastic</td>
<td>3</td>
</tr>
<tr>
<td>Triadimefon</td>
<td>DMI</td>
<td>G</td>
<td>Acropetal penetrant</td>
<td>Xylem mobile</td>
<td>Apoplastic</td>
<td>3</td>
</tr>
<tr>
<td>Triticonazole</td>
<td>DMI</td>
<td>G</td>
<td>Acropetal penetrant</td>
<td>Xylem mobile</td>
<td>Apoplastic</td>
<td>3</td>
</tr>
<tr>
<td>Propiconazole</td>
<td>DMI</td>
<td>G</td>
<td>Acropetal penetrant</td>
<td>Xylem mobile</td>
<td>Apoplastic</td>
<td>3</td>
</tr>
<tr>
<td>Tebuconazole</td>
<td>DMI</td>
<td>G</td>
<td>Acropetal penetrant</td>
<td>Xylem mobile</td>
<td>Apoplastic</td>
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</tr>
<tr>
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<td>C2</td>
<td>Acropetal penetrant</td>
<td>Xylem mobile</td>
<td>Apoplastic</td>
<td>7</td>
</tr>
<tr>
<td>Triadimefon</td>
<td>SDHI</td>
<td>C2</td>
<td>Acropetal penetrant</td>
<td>Xylem mobile</td>
<td>Apoplastic</td>
<td>7</td>
</tr>
<tr>
<td>Fluxapyroxad</td>
<td>SDHI</td>
<td>C2</td>
<td>Acropetal penetrant</td>
<td>Xylem mobile</td>
<td>Apoplastic</td>
<td>7</td>
</tr>
<tr>
<td>Polyoxin-D</td>
<td>Polyoxin-D</td>
<td>H</td>
<td>Local penetrant</td>
<td>Translaminar</td>
<td>Apoplastic</td>
<td>19</td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>Chloronitriles</td>
<td>M</td>
<td>Contact</td>
<td>…</td>
<td>…</td>
<td>M</td>
</tr>
<tr>
<td>Iprodione</td>
<td>Dicarboximide</td>
<td>E</td>
<td>Local penetrant</td>
<td>Translaminar</td>
<td>Uncertain</td>
<td>2</td>
</tr>
</tbody>
</table>

$^z$ FRAC (Fungicide Resistance Action Committee) code, active ingredients with the same code belonging to the same MOA group and target the fungus at the same metabolic site.
Chapter 2. Characterization and pathogenicity of *Rhizoctonia* spp. associated with leaf and sheath spot of bermudagrass

Abstract

Twenty isolates of a *Rhizoctonia* spp. resembling *R. zeae* were obtained from three golf courses with bermudagrass greens that were exhibiting mini-ring symptoms in 2013 and 2014. Characterization by morphological and molecular techniques showed that 17 isolates were tentatively identified as *R. zeae* and 3 were tentatively identified as *W. circinata var. zeae* based on sequences present in the GenBank database. Cluster analysis based on rDNA ITS sequences separated isolates collected in 2013 and 2014. Pathogenicity of the 20 isolates was determined by inoculating hybrid bermudagrass ‘Champion’. Inoculated plants were incubated in a growth chamber at 34/30 °C (day/night) and sealed in a closed container to ensure 100% humidity. After seven days, treatments were evaluated for disease on a scale of 0 to 10 with 10 equal to totally blighted grass. All isolates were pathogenic to the hybrid bermudagrass cultivar ‘Champion’ with mean disease severity that differed among isolates. These results demonstrated that *R. zeae* is a pathogen of hybrid bermudagrass under the imposed environmental conditions.

Introduction

The genus *Rhizoctonia* represents a diverse fungal species complex and contains more than 100 species (Ogoshi 1987). Several of these species are pathogens on turfgrasses of multiple grass species. Isolates of the multinucleate species *R. solani* Kühn, *R. oryzae* Ryker & Gooch, *R. zeae* Voorhees and *Waitea circinata var. circinata* have been collected from turfgrasses (Burpee and Martin 1992). Brown patch, caused by *R. solani*, occurs on cool-season and warm-season turfgrasses (Elliott 1999). Disease of turfgrasses caused by
fungi resembling *R. solani* can occur under conditions cooler (10 - 20 °C) than “normal” (21 - 30°C) for brown patch. The pathogen most commonly recovered from diseased grass during cool weather is *R. cerealis (=Ceratobasidium cereale)*, and the name “yellow patch” is generally used for this disease (Martin, Campbell et al. 1983, Haygood and Martin 1990,). When temperatures are 32°C or greater, *R. zeae* is often the pathogen most commonly isolated from symptomatic tissue (Martin and Lucas 1983, Leiner and Carling 1994). Toda et al. (2005) identified *Waitea circinata var. circinata* on creeping bentgrass exhibiting symptoms of brownish yellow rings and named the disease brown ring patch. The disease was identified in the US on annual bluegrass in 2007 (de la Cerda et. al, 2007).

Patches are tan to yellow-brown and range from 10 to 50 cm in diameter. The pathogen is active when daytime temperatures are between 18 – 29 °C; optimal temperature for hyphal growth was 28 °C. Few fungicides are labeled for control of brown ring patch, but polyoxin-D zinc salt, azoxystrobin, and flutolanil also provide high levels of brown ring patch control (McDonald et al. 2014). Applications of nitrogen were found to reduce disease severity and the plant growth regulator Primo Maxx (trinexapac-ethyl, Syngenta) had little effect on disease severity (Toda, Mushika et al. 2005, Wong, Chen et al. 2009).

After two hot and dry summers in 2010 and 2011, many golf course superintendents started the conversion process from creeping bentgrass to ultradwarf bermudagrass cultivars (‘Tifeagle, Champion, and Mini-verde’) on greens and other areas on golf courses. These cultivars were developed to have improved agronomic and playability characteristics when compared to ‘Tifdwarf’ – a formerly widely used cultivar. Cultivars have been developed to be an ideal sports turf – excellent wear tolerance, good recovery potential and responsiveness to nitrogen (Hanna et. al 2013). The outbreak of leaf and sheath spot began to be more
frequent after the introduction and use of ultradwarf bermudagrass. The first two bermudagrass cultivars that revolutionized golf around the world were ‘Tiflawn’, an excellent grass but too coarsely textured for greens, and ‘Tifgreen’, widely used on greens, and became very common on many golf courses in the South (Inguagiato and Martin 2015). Nine years later in 1965, a new cultivar, ‘Tifdwarf’, believed to be a natural mutation from ‘Tifgreen’ was officially released, having shorter leaves, stems and tolerance to low mowing height (Inguagiato and Martin 2015). ‘Tifdwarf’ remained the standard for bermudagrass putting greens for many years until replaced by ultradwarf cultivars in 1990s. Ultradwarf cultivars including ‘Champion’, ‘Mini-Verde’, and ‘Tifeagle’ can tolerate very close mowing of 0.23 cm, a height frequently used on greens. Due to the lower mowing heights that ultradwarfs can tolerate and the sterility of ultradwarfs, numerous southern golf courses have switched to ultradwarfs for greater green speeds (Inguagiato and Martin 2015). Symptoms of a new disease have been observed on ultradwarf bermudagrass putting greens in North Carolina, South Carolina, Georgia, Mississippi, Alabama, Louisiana, and Texas (Inguagiato and Martin 2015). The use of ultradwarf bermudagrass cultivars, reduced nitrogen fertility, nutrient deficiency due to sand based greens, and high demand on low mowing height for fast green speed have contributed to the outbreak of Rhizoctonia leaf and sheath spot (Inguagiato and Martin 2015). Recently, golf course superintendents from North Carolina reported leaf and sheath spot on bermudagrass putting greens in summer every year. This particular disease seems similar in etiology and epidemiology to brown ring patch on cool-season turfgrasses. 

*Rhizoctonia* leaf and sheath spot (RLSS) primarily affects warm-season turfgrasses, especially when conditions are warm, humid and cloudy. Isolations from affected turfgrasses demonstrated a constant association with *R. zeae* (Elliott 1999). The initial symptom on
warm-season grasses is leaf blight that can lead to large areas of dead turf if left uncontrolled. Typically RLSS was considered a minor issue on most warm-season grasses because it seems to be most problematic on turfgrass swards that receive little inputs such as bowling greens and croquet courts (Martin, Campbell et al. 1983, Elliott 1999). However, with the widespread acceptance of bermudagrass cultivars specially developed for putting greens this disease has become more problematic. The isolates collected from bermudagrass were shown to the pathogenic, but they were most aggressive towards cool-season hosts (Martin Jr and Lucas 1983, Martin, Campbell et al. 1983).

Although the disease caused by R. zeae has been reported many times all over the world on turfgrass (Elliott 1999, Mitkowski 2003, Vajna and Oros 2005), pathogenicity and molecular characterization of these isolates associated with ultradwarf bermudagrasses remains unclear. Golf course superintendents did not have an effective management strategy for RLSS. Some superintendents implemented an integrated pest management strategy by increasing mowing height and incorporating topdressing and rolling, a lesson learned from previous research on cool-season greens (Inguagiato and Martin 2015). Therefore the objectives of this study were to 1) characterize morphologically and molecularly isolates of a pathogen resembling R. zeae obtained from symptomatic ultradwarf bermudagrass, and 2) evaluate the pathogenicity of the pathogen on common cultivars of hybrid ultradwarf bermudagrass.

**Materials and method**

**Isolation and long-term storage.** Twenty isolates of a pathogen resembling R. zeae were obtained from golf courses with bermudagrass greens exhibiting ring symptoms in 2013 and 2014 (Table 2.1). Grass cores were taken at the margin of characteristic rings on greens.
Rings were yellow to orange in color and typically were only 12 to 24 cm in diameter. Plant tissue was rinsed under tap water for 5 minutes to remove soil and debris, soaked in 70% ethanol for 3 minutes, rinsed with sterile water, and then blotted dry on Kimwipes. Four pieces of leaf tissue were plated on one Petri dish containing potato dextrose agar (PDA, Difco). Following 48 hours of incubation in dark at 23 °C, hyphal tips of mycelium with characteristics of *R. zeae* (right-angled branch, septa above the branch, and hyphae with oily spots), were transferred to PDA. For long-term storage, a plug taken from margin of mycelium was put mycelium-side down on the center of PDA plate covered with several filter paper pieces. After incubation in dark for 72 hours, filter papers were fully covered with fungus mycelium, were removed and stored in small envelopes in a -80 °C freezer.

**Morphological and Molecular characterization.** Isolates were transferred to Petri dishes containing 10 ml PDA and maintained at 23°C in the dark. Width of hyphae was determined after 3 days using and ocular micrometer. Cultures were then allowed to develop sclerotia at room temperature without supplemental lightening. Culture morphology was documented and sclerotia were characterized according to shape, size and color.

For DNA extraction, each isolate was grown on cellophane-covered PDA. Mycelium was scraped from the cellophane with a scalpel and transferred to 1.5 mL micro-centrifuge tubes. After adding 2 scoops of autoclaved glass beads and solution A (Easy DNA Kit, Invitrogen Corp., K1800-01), the tube was vortexed for 10-15 seconds, disc-vortexed for 2 min, and then incubated at 65 °C for 15 min. After 15 min, solution B and chloroform were added, centrifuged for 15 min, and the upper phase was added to ethanol diluted mussel glycogen for precipitation. And suspended residue in 50 µL TE buffer for incubation overnight at 4°C. Add 2.5 µL RNase to tube and incubate at 37°C for 45 min, quantify DNA
by Nanodrop (model ND-1000, software v3.8.1) at 260 nm, and then standardized to 50 ng/µL.

Reaction volume was 15 µL and consisted of 10 × buffer with MgCl₂, 0.5 mM dNTPs, 0.5 µM each primer, 0.0027 U of Taq polymerase (Invitrogen Corp., Carlsbad, CA), and 50 ng of genomic DNA. The thermal cycle contains an initial denaturation step at 95°C for 3 min, followed by 33 cycles of 95°C for 30 sec, 58 °C for 1 min, and 72 °C for 45 sec, and a final extension step at 72 °C for 2 min. DNA genomic sequencing was achieved by using Big Dye Buffer, ITS 4 or ITS 5 primer, Big Dye v3.1 on a 3730xl DNA Analyzer capillary system (Applied Biosystems) at the Duke University Institute for Genomic Sciences and policy (Durham, NC). Reads were compared with deposited sequences in GenBank by BLAST. A phylogenetic tree was constructed in CLC Main Workbench by method UPGMA, and genetic distance was calculated by the Kimura 80 model. Bootstrap values are indicated adjacent to the nodes based on 1,000 resamplings of the data set.

**Pathogenicity.** Cone-tainers, 4-cm diam x 20.5-cm deep, containing 85% sand and 15% peat were planted with ‘Champion’ bermudagrass cores, 2-cm diam, gathered from Lake Wheeler Turfgrass Field Lab in Raleigh, NC. After collection, cores were washed free of soil and planted. The cone-tainers were placed in a phytotron growth chamber and grown for 8 weeks at 33°C/30°C day/night with a 12-h photoperiod. Grass was trimmed weekly to 2.5-cm tall. The turf was maintained in the chamber by daily mist irrigation to provide adequate leaf wetness for infection and disease development, and a nutrient solution containing 106.23 mg/L nitrogen, 10.41 mg/L phosphorus, 111.03 mg/L potassium, 54.40 mg/L calcium, 12.40 mg/L magnesium, 5.00 mg/L iron, 13.19 mg/L sulfur, 0.113 mg/L
manganese, 0.24 mg/L boron, 0.013 mg/L zinc, 0.005 mg/L copper, 0.0003 mg/L cobalt, 0.005 mg/L molybdenum, and 11.04 mg/L sodium was applied every other day.

The hybrid bermudagrass in each cone-tainer was inoculated with three PDA plugs fully covered with mycelium. The inoculum was prepared by placing a 6-mm plug collected from an actively growing culture in the center of a Petri dish containing PDA. After 3-days in the dark at 23 °C, inoculum was cut from the margin of fresh mycelium, and then placed mycelium side down on the turf surface. Three 6-mm plugs of fungal free PDA were placed on turfgrass plants to serve as non-inoculated controls. Cone-tainers were placed in a box with 2-inches of water at the bottom and covered with a plastic lid to maintain 100% humidity, and mist irrigated daily with DI water. The humidity chambers were in a growth chamber at the NCSU Phytotron at 30-33 °C with a 12-hour photoperiod and arranged in a completely random design with 3 replicates per isolate and 3 control cone-tainers. After growing for 7 days, disease severity was visually estimated using a 0 to 10 scale, with 0 = no symptom, 10 = completely blighted. The entire experiment was repeated twice.

**Data analyses.** All data were analyzed by SAS (version 9.4; SAS Inc., Cary, NC). Analysis of variance was performed by PROC GLM to compare the disease severity difference between isolate and experiments. Fisher’s Protected LSD was used to compare disease severity difference among isolates.

**Results**

**Morphological Identification of putative R. zeae isolates.** Seven and 13 isolates of *R. zeae* were obtained in the fall of 2013 of 2014, respectively. Three isolates were collected from cv. ‘Mini-verde’ and the remaining 17 isolates were sampled from cv. ‘Champion’. All isolates had vegetative hyphae 3 to 6 µm in width, had right-angle branching, and produced
white to buff-colored colonies with increasing age (after 60 days) (Figure 2.1). After two to three weeks of incubation, 12 isolates produced orange, salmon-colored spherical sclerotia immersed in the PDA, while the other 8 isolates produced sclerotia in the medium that were spherical and dark brown (Table 2.1).

**Phylogenetic analysis using ribosomal DNA sequences.** ITS1, 5.8S, and ITS2 regions of the ribosomal DNA (rDNA) were amplified by PCR using primers ITS4 and ITS5 (Innis, Gelfand et al. 2012). ITS sequence data indicated that 17 isolates were *R. zeae* and 3 were *W. circinata var. zeae*. A phylogenetic tree, based on the UPGMA method, gave three clusters. Five of seven isolates collected in 2013 were clustered with isolates obtained from cool season turfgrass (based on 100% bootstrap), and 10 of 13 isolates collected in 2014, two isolates obtained in 2013, and the *W. circinata* isolates made an individual clade, while 3 other isolates collected in 2014 made another clade; they all clustered with *Waitea circinata* isolates from GenBank (Fig. 2.2).

**Pathogenicity of *R. zeae* isolates to bermudagrass.** All 20 isolates were evaluated for pathogenicity on ultradwarf bermudagrass. Isolates performed similarly across both runs of the experiment, so data were combined for analysis and presentation.

After 7 days of incubation in the growth chamber, all 20 isolates were pathogenic to ‘Champion’. Leaf blight was observed by two days after inoculation; discrete leaf lesions were not observed during the experiment. Across all twenty isolates in the two trials, disease severity ranged from 2.17 (~21.7% disease) to 5 (~50% disease), with a mean severity of 3, 30% of leaf blades blighted; whereas, in the control plants exhibited only 1.7% blighted leaf tissue. Seventeen of the 20 isolates caused disease, with no difference in level of disease among the 17 isolates, regardless of host or geographic origin.
Discussion

Brown patch, caused by *R. solani*, is a major disease of turfgrass, with ideal temperatures for disease development are between 21°C to 25°C (Kammerer and Harmon 2008). When air temperatures are higher (>32°C), *R. zeae* was frequently isolated from grasses with patch symptoms. On tall fescue, *R. zeae* was isolated from areas showing dark gray-brown or yellow arcs or circles (Martin Jr and Lucas 1983, Haygood and Martin 1990, Burpee and Martin 1992). This study describes the presence of *R. zeae* leading to RLSS on ultradwarf bermudagrass in NC and AL based on morphological and genetic characterization. Isolates fit both the morphological and phylogenetic characteristics of *R. zeae*. *R. zeae* can be easily distinguished from *R. solani* by the white to buff colored mycelium compared to more brown hyphae for *R. solani*, and can be distinguished from *Waitea circinata* by the morphology of the sclerotia (Burpee and Martin 1992). *W. circinata* is known as the teleomorph of *R. zeae* and *R. oryzae*. This fungus was classified into three varieties, *W. c. var. oryzae*, *W. c. var. zeae* and *W. c. var. circinata*, based on differences in morphology of their vegetative state. The anamorphic name of *W. c. var. circinata* has not been assigned yet, but the classification of these three varieties has been supported by molecular analysis and by whole-cell fatty acid analysis (Toda, et al. 2005, Toda et al. 2007, Priyatmojo 2002). In this study, the sclerotia of collected *R. zeae* isolates turned from orange to dark brown, but remained orange in some cases, corresponding with research done by Garcia et al. and Leiner and Carling (Leiner and Carling 1994, González García, Ramos Ramos et al. 2012).

Phylogenetic sequencing was used to distinguish species of *Rhizoctonia* and varieties of *W. circinata* (Leiner and Carling 1994, de la Cerda, Douhan et al. 2007, González García, Ramos Ramos et al. 2012). Isolates can be identified based on the similarity of conserved
sequences such as ITS1, the 5.8S ribosomal subunit, and ITS2 when teleomorph status is not found (Kammerer, Burpee et al. 2011). A phylogenetic tree based on UPGMA indicated that our isolates fell into three clades, isolates that clustered with *R. zeae* were collected from cool-season grass and produced orange, salmon-colored sclerotia, while isolates that were clustered with *W. circinata* produced dark brown sclerotia.

The situation is complicated when considering the nomenclature of this pathogen. Leiner and Carling (Leiner and Carling 1994) proposed that the anamorphs of *W. circinata* now known as *R. zeae*, *R. circinata* and *R. oryzae* would be designated as *R. circinata var. oryzae*, *R. circinata var. circinata* and *R. circinata var. zeae*, respectively. This taxonomic system would easily allow for expansion if more groups of *W. circinata* were found. Nevertheless, de la Cerda et al. held the opinion that a strong precedent was already established for using *R. zeae* and *R. oryzae* as the anamorphic names for *W. circinata*, and since the teleomorphic state of *W. circinata* is not found readily on symptomatic turfgrass, there should be an emphasis on the use of anamorphic names. In addition, each anamorphic state of the varieties of *W. circinata* has distinct morphologies, fatty acid composition, differences in host range and distinct rDNA-ITS-region sequences. Therefore, the older names e.g., *R. zeae* and *R. oryzae*, could be maintained as anamorphic names for *W. circinata var. zeae* and *W. circinata var. oryzae* (de la Cerda, Douhan et al. 2007). Further research with detailed examination and review of *W. circinata* species is needed to formally justify a name change.

Regardless of nomenclature, *R. zeae* was identified as the pathogen that causes mini ring on hybrid bermudagrass. The isolates appeared to be insensitive to some fungicides used for patch control (Elliott 1999, Martin, Lucas et al. 1984). For several years until the mid-
1970s, *R. solani* was the only fungus associated with turfgrass (Inguagiato and Martin 2015). To date, *R. zeae* causing mini ring symptoms on hybrid bermudagrass at high temperature range is more common. Pathogenicity was evaluated at high temperature range (34/30 °C, day/night) since *R. zeae* was reported to be more aggressive in warm conditions (Martin Jr and Lucas 1983). Although some papers confirmed pathogenicity of *R. zeae* isolates on cool-season grass, corn, wheat, cotton, and soybean (Sumner and Bell 1982, Tomaso-Peterson and Trevathan 2007, Pańka, West et al. 2013), only Elliot reported pathogenicity of *R. zeae* isolates collected from Ohio and Florida on hybrid bermudagrass. Consistent with this study, the initial symptom was leaf blight (Haygood and Martin 1990, Elliott 1999). This was the first research to document the pathogenicity of *R. zeae* associated with RLSS symptoms on ultradwarf bermudagrass. Rings were not observed, but the plant cultures were likely too small for an observable ring to appear. Future studies should focus on field inoculations to reproduce the ring symptom associated with this pathogen on golf course putting greens. However, blighting is observed when ultradwarf bermudagrass is challenged with *R. zeae*. Future research is needed to test pathogenicity of *R. zeae* to ultradwarf bermudagrass at different temperatures so that management can be most effective while managing this foliar disease. More research is also needed to determine the pathogenicity of *R. zeae* to different cultivars of ultradwarf bermudagrass.
Literature Cited


Table 2.1 Geographic origin, host, collection date, and hyphal diameter of 20 isolates with morphological characteristics of *Rhizoctonia zeae* used in this study.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Origin</th>
<th>Host</th>
<th>Date collected</th>
<th>Sclerotia size/color</th>
<th>ID</th>
<th>Hyphal diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>B34PA-1</td>
<td>Tarboro, NC</td>
<td>Champion</td>
<td>10/14/13</td>
<td>&lt;1mm/ Salmon</td>
<td></td>
<td>R. zeae</td>
</tr>
<tr>
<td>B34PC-1</td>
<td>Tarboro, NC</td>
<td>Champion</td>
<td>10/14/13</td>
<td>&lt;1mm/ Salmon</td>
<td></td>
<td>R. zeae</td>
</tr>
<tr>
<td>B34PH-1</td>
<td>Tarboro, NC</td>
<td>Champion</td>
<td>10/14/13</td>
<td>&lt;1mm/ Salmon</td>
<td></td>
<td>R. zeae</td>
</tr>
<tr>
<td>30PC-1</td>
<td>Tarboro, NC</td>
<td>Champion</td>
<td>10/14/13</td>
<td>&lt;1mm/ Salmon</td>
<td></td>
<td>R. zeae</td>
</tr>
<tr>
<td>KHSITD</td>
<td>Lillington, NC</td>
<td>Mini Verde</td>
<td>10/14/13</td>
<td>&lt;1mm/ Salmon</td>
<td></td>
<td>R. zeae</td>
</tr>
<tr>
<td>KH1-2</td>
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<td>Mini Verde</td>
<td>10/14/13</td>
<td>&lt;1mm/ Salmon</td>
<td></td>
<td>R. zeae</td>
</tr>
<tr>
<td>KH3-1</td>
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<td>Mini Verde</td>
<td>10/14/13</td>
<td>&lt;1mm/ Salmon</td>
<td></td>
<td>R. zeae</td>
</tr>
<tr>
<td>19790-1</td>
<td>Opelika, AL</td>
<td>Champion</td>
<td>10/21/14</td>
<td>&lt;1mm/ dark brown</td>
<td></td>
<td>R. zeae</td>
</tr>
<tr>
<td>19790-2</td>
<td>Opelika, AL</td>
<td>Champion</td>
<td>10/21/14</td>
<td>&lt;1mm/ dark brown</td>
<td></td>
<td>R. zeae</td>
</tr>
<tr>
<td>19790-3</td>
<td>Opelika, AL</td>
<td>Champion</td>
<td>10/21/14</td>
<td>&lt;1mm/ dark brown</td>
<td></td>
<td>R. zeae</td>
</tr>
<tr>
<td>19790-4</td>
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<td>Champion</td>
<td>10/21/14</td>
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<td>R. zeae</td>
</tr>
<tr>
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<td>Champion</td>
<td>10/21/14</td>
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<td>R. zeae</td>
</tr>
<tr>
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<td>Champion</td>
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<td>R. zeae</td>
</tr>
<tr>
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<td>10/21/14</td>
<td>&lt;1mm/ dark brown</td>
<td></td>
<td>R. zeae</td>
</tr>
<tr>
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<td>Opelika, AL</td>
<td>Champion</td>
<td>10/21/14</td>
<td>&lt;1mm/ dark brown</td>
<td></td>
<td>R. zeae</td>
</tr>
<tr>
<td>THMR1</td>
<td>N.A.</td>
<td>Champion</td>
<td>11/06/14</td>
<td>&lt;1mm/ salmon</td>
<td></td>
<td>R. zeae</td>
</tr>
<tr>
<td>MCMR1</td>
<td>N.A.</td>
<td>Champion</td>
<td>11/06/14</td>
<td>&lt;1mm/ orange</td>
<td></td>
<td>W. c. var. zeae</td>
</tr>
<tr>
<td>MCMR2</td>
<td>N.A.</td>
<td>Champion</td>
<td>11/06/14</td>
<td>&lt;1mm/ orange</td>
<td></td>
<td>W. c. var. zeae</td>
</tr>
<tr>
<td>MCMR3</td>
<td>N.A.</td>
<td>Champion</td>
<td>11/06/14</td>
<td>&lt;1mm/ orange</td>
<td></td>
<td>W. c. var. zeae</td>
</tr>
<tr>
<td>NHTMR1</td>
<td>N.A.</td>
<td>Champion</td>
<td>11/06/14</td>
<td>&lt;1mm/ orange</td>
<td></td>
<td>R. zeae</td>
</tr>
</tbody>
</table>
Fig. 2.1. Mycelia and sclerotia characteristics and colony morphology of *Rhizoctonia zeae* (*W. circinata* var. *zeae*). A, *Rhizoctonia zeae* dark brown and spherical sclerotia. B, *R. zeae* right-angle branched mycelia and septa. C, *R. zeae* colony morphology and three colors of sclerotia, salmon, dark brown, and orange (left to right).
Fig. 2.2. UPGMA phylogram of *Rhizoctonia zeae* isolates produced from sequences of rDNA regions ITS1, 5.8S, and ITS2. Scale bar presents horizontal distance corresponding to genetic distance as calculated by the Kimura 80 model. Bootstrap values are indicated adjacent to the nodes based on 1,000 resamplings of the data set. Branches with bootstrap value greater than 80 were highlighted. GenBank species are named with two letters followed by six numbers. Isolates provided by Alyssa collected from cool-season turfgrass names start with “AL_”.
Table 2.2 Pathogenicity of 20 “R. zeae” isolates on ultradwarf bermudagrass

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Disease severity&lt;sup&gt;y&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>KHSITD</td>
<td>5&lt;sup&gt;A-Z&lt;/sup&gt;</td>
</tr>
<tr>
<td>30PC-1</td>
<td>4.33&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>19790-4</td>
<td>4&lt;sup&gt;A-C&lt;/sup&gt;</td>
</tr>
<tr>
<td>19790-8</td>
<td>4&lt;sup&gt;A-C&lt;/sup&gt;</td>
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<tr>
<td>19790-6</td>
<td>3.67&lt;sup&gt;A-D&lt;/sup&gt;</td>
</tr>
<tr>
<td>19790-7</td>
<td>3.5&lt;sup&gt;A-D&lt;/sup&gt;</td>
</tr>
<tr>
<td>19790-9</td>
<td>3.17&lt;sup&gt;A-E&lt;/sup&gt;</td>
</tr>
<tr>
<td>THMR1</td>
<td>3.17&lt;sup&gt;A-E&lt;/sup&gt;</td>
</tr>
<tr>
<td>NHTMR1</td>
<td>3.17&lt;sup&gt;A-E&lt;/sup&gt;</td>
</tr>
<tr>
<td>B34PH-1</td>
<td>3.17&lt;sup&gt;A-E&lt;/sup&gt;</td>
</tr>
<tr>
<td>19790-2</td>
<td>2.83&lt;sup&gt;B-E&lt;/sup&gt;</td>
</tr>
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<td>19790-1</td>
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<tr>
<td>KH1-2</td>
<td>2.5&lt;sup&gt;B-E&lt;/sup&gt;</td>
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<tr>
<td>MCMR3</td>
<td>2.33&lt;sup&gt;C-E&lt;/sup&gt;</td>
</tr>
<tr>
<td>B34PC-1</td>
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</tr>
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<td>MCMR1</td>
<td>2.17&lt;sup&gt;C-E&lt;/sup&gt;</td>
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<tr>
<td>MCMR2</td>
<td>2.17&lt;sup&gt;C-E&lt;/sup&gt;</td>
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<td>19790-3</td>
<td>1.83&lt;sup&gt;D-F&lt;/sup&gt;</td>
</tr>
<tr>
<td>KH3-1</td>
<td>1.83&lt;sup&gt;D-F&lt;/sup&gt;</td>
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<tr>
<td>B34PA-1</td>
<td>1.33&lt;sup&gt;EF&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>0.17&lt;sup&gt;F&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>x</sup> Data was from two experiments combined for analysis.
<sup>y</sup> Disease severity was determined after 7 days of inoculation and based on a 0 to 10 scale where 0=no disease, 10=all leaves turned blight. Percentage of diseased leaf area.
<sup>z</sup> Values label with same letter are not statistically significant different with each other based on Fisher’s Protected LSD
Chapter 3. *In vitro* Sensitivity of *Rhizoctonia zeae* to Fungicides and Evaluation of Fungicide Applications for Control of *Rhizoctonia* Leaf and Sheath Spot on Golf Greens

Abstract

Twenty isolates of *Rhizoctonia zeae* were collected from ultradwarf bermudagrass exhibiting symptoms of Rhizoctonia leaf and sheath spot from two locations in North Carolina. Seven growth chambers, with temperature settings at 10, 15, 20, 25, 30, 35, and 40 °C, were used to evaluate the effects of temperature on the radial growth of 7 isolates collected in 2013. Sensitivity of 7 isolates collected in 2013 to 16 fungicides (flutolanil, pyraclostrobin, fluoxastrobin, azoxystrobin, propiconazole, triticonazole, triadimefon, penthiopyrad, fluxapyroxad, azoxystrobin+difenoconazole, tebuconazole, difenoconazole, iprodione, polyoxin-D zinc salt, fluxapyroxad+pyraclostrobin, and chlorothalonil) was tested. The sensitivity of 13 isolates collected in 2014 was tested to 5 fungicides (chlorothalonil, azoxystrobin, pyraclostrobin, difenoconazole, and flutolanil). The efficacy of 9 fungicides (propiconazole, triticonazole 30.1%, triticonazole 19.2%, tebuconazole, zoxystrobin + difenoconazole, trifloxystrobin + triadimefon, pyraclostrobin + fluxapyroxad, polyoxin-D zinc salt, iprodione + trifloxystrobin) for control of *Rhizoctonia* leaf and sheath spot (RLSS) was evaluated in 2014 and 2015 on two different golf greens in NC. In general, all the isolates were extremely sensitive to DMI and SDHI fungicides (EC$_{50}$ < 1 mg a.i. L$^{-1}$). Tebuconazole was the most effective fungicide in suppressing growth of *R. zeae* and they were moderately sensitive to iprodione and chlorothalonil. All isolates showed insensitivity to QoI fungicides (EC$_{50}$ > 10 mg a.i. L$^{-1}$). In 2014, disease symptoms were first observed in early July on golf greens. Only applications of tebuconazole, 30.1% triticonazole, and 19.2% triticonazole reduced RLSS severity compared to the untreated control. In 2015, turfgrass
quality differences were observed after first fungicide application, plots where fungicides were applied had better turf quality than control plots. After a second application, a decrease in turf quality of plots treated with DMI was detected as compared to the untreated control. Plots treated with non-DMI fungicides showed better turf quality than the untreated control, but none of them were statistically different from untreated control.

Introduction

The genus *Rhizoctonia* represents diverse fungal species complex containing more than 100 species (Ogoshi 1987). Isolates of multinucleate species, *R. solani* Kühn, *R. oryzae* Ryker & Gooch, *R. zeae* Voorhees and *Waitea circinata var. circinata*, have been reported from cool- and warm-season turfgrasses (Burpee and Martin 1992). *W. circinata var. circinata*, was first reported as the causal agent of brown ring patch by Toda et al., in 2005. They recovered the organism from creeping bentgrass with tan to yellow-brown small patches ranging from 10 to 50 cm in diameter. The pathogen was active when daytime temperatures were 18 – 29 °C. Application of nitrogen was shown to reduce disease severity, but applications of the plant growth regulator Primo Maxx (trinexapac-ethyl, Syngenta) had little effect on disease severity (Toda, Mushika et al. 2005, Wong, Chen et al. 2009). As a relatively new disease, very few fungicides are labeled for control of brown ring patch. The fungicides polyoxin-D zinc salt, azoxystrobin, and flutolanil provides high levels of brown ring patch control (McDonald et al. 2014).

*R. solani* is the most-studied fungus and is recognized as one of the major causal agents of foliar diseases on turfgrasses (Martin Jr and Lucas 1983, Martin, Lucas et al. 1984). It was found that the ideal temperatures for brown patch symptom development fall between 21 °C and 25 °C (Kammerer and Harmon 2008). The diversity of species within
Rhizoctonia allows for isolation of other species when conditions are not suitable for *R. solani*. For example, under cool, wet conditions (10 – 20 °C) *R. cerealis* (=*Ceratobasidium cereale*) is isolated from patches with a bright yellow color. This disease was termed yellow patch. In contrast, when temperatures increased above 32 °C, *R. zeae* is frequently isolated from tall fescue and from creeping bentgrass exhibiting symptoms of brown patch (Martin, Campbell et al. 1983, Haygood and Martin 1990).

Elliott first observed *R. zeae* as the causal agent of *Rhizoctonia* leaf and sheath spot (RLSS) on hybrid bermudagrass (Elliott 1999). Bermudagrass (*Cynodon dactylon*) as a C₄ plant is widely used on golf greens in transition zones. This long-term stable and uniform grass has become the primary grass used in the sports-turf industries and is rapidly gaining acceptance for golf course putting surfaces (Hanna, Raymer et al. 2013). Ultradwarf cultivars such as ‘Tifeagle, Champion, and Mini-Verde’ tolerate extremely lowing mowing heights and being a C₄ plant endures the summer stress present in the transition zone better than creeping bentgrass (Inguagiato and Martin 2015). Marketing claims surrounding these grasses indicated that fewer cultural and pesticide inputs were required to maintain comparable ball roll uniformity and distances compared to creeping bentgrass surfaces, and turfgrass managers switched from creeping bentgrass to ultradwarf bermudagrasses. The major claim was the switch would result in significant monetary savings for the golf club. Yet ultradwarf bermudagrass has a shallower root system than previous hybrid cultivars and creeping bentgrass, which predisposes them to pathogens that attack when grasses are growing slowly. This is further exacerbated because golf course putting green root zones are designed to move water through and have limited nutrient holding capacities (Inguagiato and Martin 2015). As these conversions quickly occurred so did an outbreak of a disease that
exhibited symptoms of tan spots ranging from 2cm to 10cm in diameter that can expand to
necrotic rings ranging from 10cm to 40cm in diameter. Symptoms typically developed from
mid-July through early-October. These symptoms have been observed on ultradwarf putting
greens in South Carolina, North Carolina, Georgia, Mississippi, Alabama, Louisiana, and
Texas, once symptoms develop, they persist throughout the winter months and turf recovery
is limited until spring-green up the following year (Inguagiato and Martin 2015). Isolations
from affected tissue indicated that a fungus resembling R. zeae was the most common
pathogen present. This was demonstrated in many states, yet the etiology of this disease
remained unclear until recently.

Many fungicides on the market are registered for controlling Rhizoctonia spp. on
turfgrass, with multiple modes of action (Carling, Helm et al. 1990, Kataria, Hugelshofer et
al. 1991, Hamada, Yin et al. 2011, Amaradasa, Lakshman et al. 2014). However, few
fungicides are labeled for control of RLSS. In an in vitro fungicide sensitivity study, all the
isolates of R. zeae were insensitive to benomyl, but were sensitive to carboxin, PCNB,
fenpropimorph, and DMI fungicides (triadimefon, hexaconazole, propiconazole, and
flusilazole) (Martin et al. 1984, Carling et. al 1990, Kataria et. al 1991), This study was
conducted prior to the release of many QoI and SDHI fungicides, and many of the products
tested have been removed from the market place or severely restricted in their use (Latin
2011). Variation in sensitivity among Rhizoctonia species to fungicides has been reported,
indicating that R. solani and R. zeae respond to fungicides differently (Martin, Lucas et al.
practices off of other more well studied Rhizoctonia diseases in turf is most likely not valid
for this particular disease. A rigorous examination of the effects of fungicides on growth of R.
zeae associated with ultradwarf bermudagrass is warranted and needed as little has been done with this pathogen in recent literature. Therefore, we selected 16 fungicides from 6 groups in 2014 (Table 3.1) and 5 fungicides from 4 groups in 2015 (Table 3.2) to test their in vitro efficacy in controlling radial-growth of *R. zeae* isolates.

The objectives of this study were to determine the sensitivity of isolates *R. zeae* from ultradwarf bermudagrasses to 16 different fungicides from six chemical groups and to determine the efficacy of fungicides on bermudagrass greens to control disease caused by *R. zeae*.

**Materials and method**

**Collection and long-term storage.** In 2013 and 2014, 7 and 13 isolates resembling *R. zeae* from two locations in North Carolina were collected, respectively (Table 3.3). Grass cores were taken at the margin of symptom rings. To isolate from symptomatic leaves, leaf blades were rinsed under tap water for 5 minutes, soaked in 70% ethanol for 3 minutes, rinsed with sterile water, and blotted dry on Kimwipes. Four pieces of leaf tissue were plated on a 9-cm diam Petri dish containing potato dextrose agar (PDA, Difco). Following 48 hours of incubation in darkness at 23 °C, hyphal tips of mycelium with right-angled branch, septa above the branch, and hyphae with oily spots, characteristics of *R. zeae* were transferred to potato dextrose agar (PDA). For long-term storage, plugs were taken from the margin of a colony were put mycelium-side down on the center of a PDA plate covered with several sterile filter paper pieces that were approximately 1 square inch. After incubation in the dark for 72 hours, filter papers that were fully covered with fungus mycelium were removed from the dish, placed in small envelopes and stored in a -80 °C freezer.
**Effect of temperature on the radial growth of *R. zeae***. Seven growth chambers, with temperature setting at 10, 15, 20, 25, 30, 35, and 40 °C, were used to evaluate the effects of temperature on the radial growth of 7 isolates collected in 2013. Plugs taken from margin of 3-day old *R. zeae* colonies were placed mycelium-side down on the surface potato dextrose agar (PDA) in Petri dishes. After incubation in darkness for 24 hours at 7 different temperatures, radial growth of 3 replications of each isolate × temperature treatment was measured in two perpendicular directions. The entire temperature study was repeated three times.

**Sensitivity test.** The sensitivity of seven *R. zeae* isolates to 16 fungicides (Table 3.1), and 13 “*R. zeae*” isolates to 5 fungicides (Table 3.2) was determined in mycelial growth assays in 2014 and 2015, respectively. Hyphal plugs (6mm) from the edge of actively growing colonies on PDA were placed in the center of Petri dishes amended with six concentrations (0, 0.001, 0.01, 0.1, 1, 10 mg L⁻¹) of commercially formulated fungicides (Table 3.1). Fungicide solutions were added to autoclaved PDA after cooling to 50 °C.

After incubation in darkness for 24, 48, and 72 hours at 23 °C, the radial growth of mycelium was measured in two perpendicular directions. In 2014, each concentration was replicated three times and the whole experiment was repeated three times. In 2015, each fungicide × isolate was replicated three times and the entire experiment was conducted twice. The percent growth inhibition for each isolate × fungicide combination was calculated using the following formula.

\[
\text{%Inhibition} = (1 - \frac{\text{diameter of treated}}{\text{diameter of control}}) \times 100
\]

**Field study experimental design.** Fungicides were evaluated for their efficacy against RLSS in field plots. Treatments consisted of propiconazole, triticonazole (30.1% and
19.2 %), tebuconazole, azoxystrobin + difenoconazole, trifloxystrobin + triadimefon, pyraclostrobin + fluxapyroxad, iprodione + trifloxystrobin and polyoxin-D zinc salt and were arranged in a randomized complete block design. A non-treated control was included in the study. During summer 2014, the experiment was conducted in Tarboro, NC on a 10-year-old ‘Champion’ bermudagrass putting green that had a history of RLSS disease in 2013. In summer of 2015, the experiment conducted at the Lake Wheeler Turfgrass Research Farm in Raleigh, NC and was conducted on a 2-year old ‘Champion’ putting green. Plots were aligned on the ‘Champion’ Ultradwarf bermudagrass green, mowing was performed six times weekly at a height of 0.37 cm and clippings were collected. This field was irrigated as needed to prevent drought stress. Rolling was performed every other week for green speed and consistency. Fertilizer was applied as 13-3-13 on 8 May, 26 May, and 18 September at the rate of 0.23 kg N/M, was applied as 46-0-0 (U flex) on 8 June, 21 July, and 17 August at the rate of 0.09, 0.11, 0.11 kg N/M respectively, was applied as 28-5-18 on 23 June and 4 August at the rate of 0.09 lb N/M, was applied as 18-0-6 on 7 July at the rate of 0.09 lb N/M. Micronutrients were applied a Harrell’s Iron Mn Mg (0.15 L/M) on 29 May. Wetting agents (Harrells fleet wetting agent, HydrOtecH, at 15 mL/M) were applied on 15 May, 15 June and 13 July. Insecticides were applied on 22 May (Acelpryn, chlorantraniliprole, at 0.06 mL/M), on 1 June and 16 June (Aloft, clothianidin+bifenthrin, at 8 mL/M).

**Inoculation method.** The plots were inoculated with three isolates (B34PA-1, 19790-3, and 19790-6) of *R. zeae* that were collected in 2013 and 2014. For rye grain inoculum preparation, 250ml of rye grain seeds, 10 ml of calcium carbonate powder and 220ml of warm water were put into each of three 1L-flasks, autoclaved 30 minutes in liquid cycle and cooled to room temperature. Two Petri dishes of 3-day old *R. zeae* cultures on PDA were
chopped into $1 \times 1$ cm$^2$, and added to each one 1L-flask with rye grain after cooling to room temperature. After 4 days of growth, 3 flasks of rye grain fully covered with mycelium were mixed together for inoculation of field plots. Each plot was inoculated with two points in the center, nails were stabbed into the turf surface to make an opening of canopy on the points, and then 10-ml of inoculated rye seeds were used to cover each opening. The plots were irrigated twice, before and after inoculation for 2 minutes. The entire inoculation process was repeated every month (30 days).

**Fungicide treatments.** Eleven active ingredients in nine fungicide products were evaluated, and fungicides were applied at curative rates (Table 3.4). Briskway, Tartan, Lexicon, and Interface contain a mixture of two active ingredients in one product. Among the nine fungicides, six fungicides contained a DMI fungicide. The active ingredients belonged to five fungicide mode-of-action (MOA) groups. Each group has a unique FRAC (Fungicide Resistance Action Committee) code, active ingredients having the same code belong to the same MOA group so they attack the pathogen at the same site.

**Field experiments.** During summer 2014, the experiment was conducted in Tarboro, NC on a 10-year-old ‘Champion’ bermudagrass putting green that had exhibited extreme RLSS symptoms in 2013. In the summer of 2015, the experiment was moved to the Lake Wheeler Turfgrass Research Lab in Raleigh, NC and was conducted on a 2-year old ‘Champion’ putting green. The plots were $3 \times 6$ foot rectangles, all the fungicides were sprayed in 610mL of water for 4 replications, equivalent to 40 mL per square meter with a CO$_2$-pressurized backpack sprayer at 40 psi using a TeeJet 9508E nozzle, no irrigation was implemented after fungicides had been applied. Turf quality was evaluated visually every three weeks on a 0 to 9 scale before fungicide application, where 0 equals brown, dead turf; 6
equals acceptable turf performance; and 9 means optimum color, perfect density and uniformity. Usually, we never see turf scoring 9 in nature. Area under disease progress curve (AUDPC) was calculated with the formula \[ \Sigma[(y_i+y_{i+1})/2][t_{i+1}-t_i], \] where \(i = 1,2,3,\ldots, n-1,\) \(y_i\) is the percent of diseased plot area, and \(t_i\) is the time of the \(i\)th rating (Miller, Soika et al. 2012).

**Data analysis.** Percent growth inhibition of different concentrations from each isolate \(\times\) fungicide were used to derive the fungicide concentration when 50% of colony inhibited (EC\(_{50}\)) by PROC REG in SAS (version 9.4, SAS Inc., Cary, NC). EC\(_{50}\) values of each isolate \(\times\) fungicide were subject to an ANOVA (PROC GLM) and means were separated by Waller-Duncan \(k\)-ratio \(t\)-test \((k = 100)\), isolates were regarded as extremely sensitive if the EC\(_{50}\) of a fungicide was less than 1 mg a.i./L (ppm), moderately sensitive if the EC\(_{50}\) of a fungicide was 1-10 mg a.i./L (ppm), and insensitive if EC\(_{50}\) greater than 10 mg a.i./L. For both field studies, statistical analyses were conducted by using SAS, disease severity and turf quality were subject to an ANOVA (PROC GLM) and means were separated by Fisher’s Protected LSD.

**Result**

**Growth rate.** The radial growth rates of 7 isolates collected in 2013 were evaluated. In all three experiments, the growth rate of \(R.\ zeae\) increased from 10 to 30°C across 7 isolates. The optimum temperature for \(R.\ zeae\) growing is 30°C (Figure 3.1). A rapid decline happened from 35 to 40 °C in all experiments across isolates. There is no mycelium growth at 10°C in experiment two, very little growth at 10°C in the other two experiments. At 40°C, all isolates stop growing in experiment one. They grew differently in experiment two, isolates “B34PA-1”, “B34PC-1”, “B34PH-1”, “30PC-1”, and “KHSITD” have larger radial growth than “KH1-2” and “KHSITD”. “30PC-1” had the largest growth rate, greater than the
other six isolates with 8.33mm of mycelium growing in 24-hour. All isolates grew faster at 10°C than they did at 40°C in experiments one and three. In experiment three, isolates had similar growth rates at 30 and 35°C.

**In vitro Sensitivity of R. zeae isolates to fungicides.** In 2014, the sensitivity of 7 isolates of *R. zeae* collected in 2013 (Table 3.3) was tested to 16 fungicides from 6 groups (Table 3.1). All isolates assembling *R. zeae* were extremely sensitive to tebuconazole, difenoconazole, propiconazole, fluxapyroxad, triticonazole, penthiopyrad and two combined fungicides -- azoxystrobin + difenoconazole and pyraclostrobin + fluxapyroxad (Figure 3.2, 3.3, and 3.4). The average EC$_{50}$ values for these fungicides were less than 1 mg a.i. L$^{-1}$. All isolates were moderately sensitive to pyraclostrobin, triadimefon, iprodione, chlorothalonil, flutolanil, fluoxastrobin, polyoxin-D, and azoxystrobin in experiment 1 (Figure 3.2). In experiment 2 and 3 (Figure 3.3, 3.4), they were moderately sensitive to pyraclostrobin, triadimefon, iprodione, chlorothalonil, and flutolanil; and insensitive to polyoxin-D, fluoxastrobin, and azoxystrobin, the average EC$_{50}$ values of these fungicides were more than 10 mg a.i. L$^{-1}$.

There was a significant difference (P < 0.001) of sensitivity to fungicides among isolates (Table 3.7). Isolate “30PC-1” was more sensitive to all the fungicides, with an average EC$_{50}$ value of 16 fungicides to “30PC-1” was 2.1583 mg a.i. L$^{-1}$, while the average EC$_{50}$ value of fungicides to other isolates were greater than 3 mg a.i. L$^{-1}$.

In 2015, the efficacy of five fungicides representing 4 MOA groups (Table 3.2) was evaluated in reducing radial growth of 13 isolates collected from two states in U.S. (Table 3.3). No difference of sensitivity among isolates was observed between the two experiments (Figure 3.5). All isolates were extremely sensitive to difenoconazole and pyraclostrobin. The
mean EC$_{50}$ values of these two fungicides were < 1 mg a.i. L$^{-1}$. The isolates were moderately sensitive to flutolanil and chlorothalonil (1 < EC$_{50}$ < 10 mg a.i. L$^{-1}$), and insensitive to azoxystrobin (EC$_{50}$ > 10 mg a.i. L$^{-1}$).

In general, all the isolates were extremely sensitive to DMI and SDHI fungicides (EC$_{50}$ < 1 mg a.i. L$^{-1}$), tebuconazole was the most effective fungicide for reducing hyphal growth of R. zeae among 16 fungicides. However, all isolates were insensitivity to QoI fungicides (EC$_{50}$ > 10 mg a.i. L$^{-1}$), but moderately sensitive to iprodione and chlorothalonil.

**Evaluation of Fungicide Application for Rhizoctonia Leaf and Sheath Spot Control in Golf Greens.** In 2014, disease symptoms were first observed in early July, fungicides were applied after the first detection of symptoms. However, the disease was not severe in the experimental area, and no treatments significantly suppressed RLSS symptoms compared to the control in this study (Figure 3.7). Moreover, differences among treatments with respect to turfgrass quality were not detected (Figure 3.6).

In 2015, before fungus inoculation and fungicides application, all plots had a turf quality rating of 6. Turfgrass quality differences were observed after first fungicides application, plots applied with fungicides had better turf quality than control plots (Figure 3.7). After the second application, a decrease in turf quality of plots treated with propiconazole, triticonazole (19.2% & 30.1%), and tebuconazole was detected (Figure 3.8) as compared to the untreated control, plots treated with non-DMI fungicides showed better turf quality than untreated control, but none of them were statistically different.

**Discussion**

The growth rate of 7 isolates of R. zeae was greatest at 30 °C, but growth declined at 35 °C. Similarly 30 °C has been reported to be optimal for R. zeae isolates collected from turf
and onion (Leiner and Carling 1994, Elliott 1999, Erper, Karaca et al. 2006, Kammerer, Burpee et al. 2011). The optimum growth rate of *R. zeae* isolates obtained from Florida and Ohio was 30°C, it then decreased at higher temperatures (Elliott 1999). In agreement with this study, Kammerer et al. tested the growth rate of teleomorph of *R. zeae – Waitea circinata var. zeae* at 15 to 40 °C, the optimum temperature for mycelium growth was 30 °C, growth rate dropped at 35 °C, only 5 mm of radial growth was detected at 40°C over 24 hours.

The isolates recovered in this study were most sensitive to the DMI fungicides (difenoconazole, triadimefon, triticonazole, propiconazole and tebuconaozle) with the lowest EC$_{50}$ observed for tebuconazole (EC$_{50}$ = 0.02 mg a.i. L$^{-1}$). DMI fungicides showed strong fungitoxic activity against all *Rhizoctonia* species, triticonazole as a second generation DMI fungicide, effectively control fungal growth of *R. solani* AG 1-IB and all varieties of *Waitea circinata* with EC$_{50}$ < 1 mg a.i. L$^{-1}$ (Carling, Helm et al. 1990, Kataria, Hugelshofer et al. 1991, Latin 2011, Amaradasa, Lakshman et al. 2014). Another DMI fungicide, triadimefon was less effective controlling *R. zeae* fungal growth (EC$_{50}$ = 3.97 mg a.i. L$^{-1}$), in contrast, *R. zeae* isolated from tall fescue was extremely sensitive to triadimefon with EC$_{50}$ < 1 mg a.i. L$^{-1}$ (Martin, Lucas et al. 1984), this may be due to the genetic difference of *R. zeae* isolates from warm- and cool-season turfgrass, such difference was approved by Elliot, a significant differences of sensitivity ($P < 0.05$) between *R. zeae* isolates from Florida vs. Ohio were observed for two of the seven fungicides evaluated (Elliott 1999). It is also reported that the sensitivity of *R.zeae* from turfgrass and rice was completely different, the study indicated, genetic or physiological differences existed among isolates from different hosts (e.g., corn, rice, and turfgrasses) (Kataria, Hugelshofer et al. 1991).
QoI fungicides have been reported to be effective in controlling *Rhizoctonia* groups for many years (Meyer, Bueno et al. 2006). However, in this study, all the *R. zeae* isolates showed insensitivity to the QoI fungicides ($EC_{50} > 10$ mg a.i. L$^{-1}$) except for pycraclostrobin ($EC_{50} = 1.19$ mg a.i. L$^{-1}$). Recent research conducted by Amaradasa et al. (2014) found nine isolates of *W. circinata var. zeae* (teleomorph of *R. zeae*) to be moderately sensitive to pycraclostrobin ($EC_{50} = 2.29$ mg a.i. L$^{-1}$), giving a result similar to this study. Several QoI fungicides have been discussed showing loss of effectiveness for some pathogens due to site-specific mutations (Vincelli and Dixon 2002, Ma, Felts et al. 2003, Amaradasa, Lakshman et al. 2014). Azoxystrobin resistance correlated with a single mutation in the cytochrome *b* (*cyt b*) gene causing a change of clycine to alanine, to date, at least eleven single or combined mutations in the cyt *b* gene have been reported to confer resistance to strobilurins in different organisms including bacteria, algae, yeasts, protozoa and animals (Ma, Felts et al. 2003). Further research confirmed that isolates with complete resistance contained a G143A mutation in the cyt *b* gene while partially resistance isolates a F129L mutation (Kerns and Tredway 2013). Perhaps these isolates have one of these mutations, but the explanation for the lack of sensitivity to azoxystrobin and trifloxystrobin in the isolates we collected remains unclear.

Some fungi are capable of using alternative respiration pathways in response to QoI fungicides in *in vitro* studies (Wise, Bradley et al. 2008). The chemical salicylhydroxamic acid (SHAM) is usually used to prevent a fungus from using an alternative pathway. To date, the ability of *Rhizoctonia* species to use alternative respiration is not well documented (Amaradasa, Lakshman et al. 2014). We conducted an initial test by adding SHAM to QoI fungicides (azoxystrobin, fluoxastrobin, and pycraclostrobin) amended petri dishes, applied to
seven representative isolates viz. B34PA-1, B34PH-1, B34PC-1, 30PC-1, KHSITD, KH1-2, and KH3-1. No difference was detected by comparing radial growth of isolates on QoI fungicides amended dishes with QoI fungicides + SHAM (data not shown). Similarly, no synergistic growth inhibition in SHAM plus pyraclostrobin amended plates was found for a *W. circinata var. zeae* isolate in a study conducted by Amaradasa et al. (2014). Therefore, SHAM was not added to QoI amended plates for fungicide evaluation.

SDHI fungicides specifically bind to the ubiquinone-binding site of the mitochondria complex II, so the consistent use of these site-specific fungicides can result in the selection of resistant fungal genotypes, it has been known for many years that mutants were resistant to flutolanil in several organisms (Avenot and Michailides 2010). However, flutolanil was reported to be effectively controlling *R. solani* fungal growth on rice *in vitro* (EC$_{50} = 0.0736 \pm 0.0331$ mg a.i. L$^{-1}$) and *in vivo* (Li, Hou et al. 2014). In this study, three SDHI fungicides have been used; all isolates collected in 2013 were extremely sensitive to penthiopyrad (EC$_{50} = 0.81$ mg a.i. L$^{-1}$) and fluxapyroxad (EC$_{50} = 0.50$ mg a.i. L$^{-1}$), moderately sensitive to flutolanil (EC$_{50} = 8.17$ mg a.i. L$^{-1}$), isolates collected in 2014 exhibited increased sensitivity to flutolanil (EC$_{50} = 1.24$ mg a.i. L$^{-1}$) than those collected in 2013. This study demonstrates that *R. zeae* associated with RLSS in ultradwarf bermudagrass is sensitive to SDHI fungicides but suppression of the fungus in plant tissue remains unclear.

Isolates of *R. zeae* were moderately sensitive to chlorothalonil and iprodione with EC$_{50} = 4.09$ and 4.92 mg a.i. L$^{-1}$ respectively, in agreement with reports on other *R. zeae* isolates sampled from turfgrass (Martin, Lucas et al. 1984, Amaradasa, Lakshman et al. 2014). Field results with these fungicides are still unclear most likely due to the sporadic nature of the disease. Based on anecdotal observations only, it appears that this disease
responds to cultural practices more than chemical fungicides. During this study, the golf course increased fertility and sand topdressing, which in turn may have reduced the severity and incidence of RLSS.

Management of this disease is complicated because it seems that the best fungicides can have negative effects on plant health. Bermudagrasses typically grow best when temperatures are at or above 26 °C and this pathogen seems to favor temperatures around 30 °C with symptoms typically not developing until mid-July or later. Based on these data, the best strategies for management appear to be applications of a DMI when temperatures increase to 30 °C and potentially a pigment could be used to mask the potential phytoxicity associated with DMI applications. Previous reports indicate that the phytoxicity observed on ultradwarf bermudagrasses maybe due to a growth regulator effect (Soika and Tredway 2009), therefore turfgrass managers suffering from this disease should not use a growth regulator in conjunction with a DMI fungicide. As the summer progresses and the day length shortens, turfgrass managers could switch to fungicides such as iprodione, chlorothalonil and/or SDHIs. Previous work in cool-season turfgrasses, indicates that cultural practices such as increasing nitrogen fertility and sand topdressing limited brown ring patch development. Therefore turfgrass managers should examine their cultural practices if they are suffering from this disease.
Literature Cited


Table 3.1. Trade name, common name, and mode of action group of fungicides used for evaluating *in vitro* sensitivity of seven isolates of *Rhizoctonia zeae* collected in 2013.

<table>
<thead>
<tr>
<th>Trade Name</th>
<th>Common Name</th>
<th>Group Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disarm</td>
<td>Fluoxastrobin</td>
<td>QoI</td>
</tr>
<tr>
<td>Insignia</td>
<td>Pyraclostrobin</td>
<td>QoI</td>
</tr>
<tr>
<td>Heritage</td>
<td>Azoxystrobin</td>
<td>QoI</td>
</tr>
<tr>
<td>Difenoconazole</td>
<td>Difenoconazole</td>
<td>DMI</td>
</tr>
<tr>
<td>Baleyton</td>
<td>Triadimefon</td>
<td>DMI</td>
</tr>
<tr>
<td>Triton FLO</td>
<td>Triticonazole</td>
<td>DMI</td>
</tr>
<tr>
<td>Banner Maxx</td>
<td>Propiconazole</td>
<td>DMI</td>
</tr>
<tr>
<td>Torque</td>
<td>Tebuconazole</td>
<td>DMI</td>
</tr>
<tr>
<td>Prostar</td>
<td>Flutolanil</td>
<td>SDHI</td>
</tr>
<tr>
<td>Velista</td>
<td>Penthiopyrad</td>
<td>SDHI</td>
</tr>
<tr>
<td>Xzemplar</td>
<td>Fluxapyroxad</td>
<td>SDHI</td>
</tr>
<tr>
<td>Affirm</td>
<td>Polyoxin-D Zinc Salt</td>
<td>Polyoxin-D Zinc Salt</td>
</tr>
<tr>
<td>Daconil W S</td>
<td>Chlorothalonil</td>
<td>Choronitriles</td>
</tr>
<tr>
<td>Iprodione</td>
<td>Iprodione</td>
<td>Dicarboximide</td>
</tr>
<tr>
<td>Briskway</td>
<td>Azoxystrobin+Difenoconazole</td>
<td>QoI +DMI</td>
</tr>
<tr>
<td>Lexicon</td>
<td>Pyraclostrobin+Fluxapyroxad</td>
<td>QoI +SDHI</td>
</tr>
</tbody>
</table>
Table 3.2. Trade name, common name, and mode of action group of fungicides used for evaluating *in vitro* sensitivity of 13 isolates of *Rhizoctonia zeae* collected in 2014.

<table>
<thead>
<tr>
<th>Trade Name</th>
<th>Common Name</th>
<th>Group Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insignia</td>
<td>Pyraclostrobin</td>
<td>QoI</td>
</tr>
<tr>
<td>Heritage</td>
<td>Azoxystrobin</td>
<td>QoI</td>
</tr>
<tr>
<td>Difenoconazole</td>
<td>Difenoconazole</td>
<td>DMI</td>
</tr>
<tr>
<td>Prostar</td>
<td>Flutolanil</td>
<td>SDHI</td>
</tr>
<tr>
<td>Daconil W stik</td>
<td>Chlorothalonil</td>
<td>Chlorontriles</td>
</tr>
</tbody>
</table>
Table 3.3. Geographic origin, host, and collection date of 20 isolates of *Rhizoctonia zeae* used in this study.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Origin</th>
<th>Host</th>
<th>Date collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>B34PA-1</td>
<td>Tarboro, NC</td>
<td>Champion</td>
<td>10/14/13</td>
</tr>
<tr>
<td>B34PC-1</td>
<td>Tarboro, NC</td>
<td>Champion</td>
<td>10/14/13</td>
</tr>
<tr>
<td>B34PH-1</td>
<td>Tarboro, NC</td>
<td>Champion</td>
<td>10/14/13</td>
</tr>
<tr>
<td>30PC-1</td>
<td>Tarboro, NC</td>
<td>Champion</td>
<td>10/14/13</td>
</tr>
<tr>
<td>KHSITD</td>
<td>Lillington, NC</td>
<td>Mini Verde</td>
<td>10/14/13</td>
</tr>
<tr>
<td>KH1-2</td>
<td>Lillington, NC</td>
<td>Mini Verde</td>
<td>10/14/13</td>
</tr>
<tr>
<td>KH3-1</td>
<td>Lillington, NC</td>
<td>Mini Verde</td>
<td>10/14/13</td>
</tr>
<tr>
<td>19790-1</td>
<td>Opelika, AL</td>
<td>Champion</td>
<td>10/21/14</td>
</tr>
<tr>
<td>19790-2</td>
<td>Opelika, AL</td>
<td>Champion</td>
<td>10/21/14</td>
</tr>
<tr>
<td>19790-3</td>
<td>Opelika, AL</td>
<td>Champion</td>
<td>10/21/14</td>
</tr>
<tr>
<td>19790-4</td>
<td>Opelika, AL</td>
<td>Champion</td>
<td>10/21/14</td>
</tr>
<tr>
<td>19790-6</td>
<td>Opelika, AL</td>
<td>Champion</td>
<td>10/21/14</td>
</tr>
<tr>
<td>19790-7</td>
<td>Opelika, AL</td>
<td>Champion</td>
<td>10/21/14</td>
</tr>
<tr>
<td>19790-8</td>
<td>Opelika, AL</td>
<td>Champion</td>
<td>10/21/14</td>
</tr>
<tr>
<td>19790-9</td>
<td>Opelika, AL</td>
<td>Champion</td>
<td>10/21/14</td>
</tr>
<tr>
<td>THMR1</td>
<td>N.A.</td>
<td>Champion</td>
<td>11/06/14</td>
</tr>
<tr>
<td>MCMR1</td>
<td>N.A.</td>
<td>Champion</td>
<td>11/06/14</td>
</tr>
<tr>
<td>MCMR2</td>
<td>N.A.</td>
<td>Champion</td>
<td>11/06/14</td>
</tr>
<tr>
<td>MCMR3</td>
<td>N.A.</td>
<td>Champion</td>
<td>11/06/14</td>
</tr>
<tr>
<td>NHTMR1</td>
<td>N.A.</td>
<td>Champion</td>
<td>11/06/14</td>
</tr>
</tbody>
</table>
Table 3.4. Trade name, common name, application rate, and group of fungicides used for evaluating efficacy in controlling RLSS (*Rhizoctonia* leaf and sheath spot) on golf greens in 2014 and 2015.

<table>
<thead>
<tr>
<th>Trade Name</th>
<th>Common Name</th>
<th>Application Rate</th>
<th>Group Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banner Maxx</td>
<td>Propiconazole</td>
<td>0.64 ml/m²</td>
<td>DMI</td>
</tr>
<tr>
<td>Triton FLO</td>
<td>Triticonazole 30.1%</td>
<td>0.35 ml/m²</td>
<td>DMI</td>
</tr>
<tr>
<td>Trinity</td>
<td>Triticonazole 19.2%</td>
<td>0.64 ml/m²</td>
<td>DMI</td>
</tr>
<tr>
<td>Torque</td>
<td>Tebuconazole</td>
<td>0.35 ml/m²</td>
<td>DMI</td>
</tr>
<tr>
<td>Briskway</td>
<td>Azoxystrobin+Difenoconazole</td>
<td>0.23 ml/m²</td>
<td>QoI + DMI</td>
</tr>
<tr>
<td>Tartan</td>
<td>Trifloxystrobin+Triadimefon</td>
<td>0.64 ml/m²</td>
<td>QoI + DMI</td>
</tr>
<tr>
<td>Lexicon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intrinsic</td>
<td>Pyraclostrobin+Fluxapyroxad</td>
<td>0.15 ml/m²</td>
<td>QoI + SDHI</td>
</tr>
<tr>
<td>Affirm</td>
<td>Polyoxin-D Zinc Salt</td>
<td>0.27 g ml/m²</td>
<td>Polyoxin-D Zinc</td>
</tr>
<tr>
<td>Interface</td>
<td>Iprodione+Trifloxystrobin</td>
<td>1.59 ml/m²</td>
<td>Chloronitriles+QoI</td>
</tr>
</tbody>
</table>
Fig. 3.1. Radial growth of seven isolates of *Rhizoctonia zeae* isolates on potato dextrose agar at different temperatures in three experiments (A, B, and C).
Table 3.5. EC\textsubscript{50} value of QoI and Polyoxin-D fungicides for isolates collected in 2013.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Fluoxastrobin</th>
<th>Pyraclostrobin</th>
<th>Azoxystrobin</th>
<th>Polyoxin-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>B34PA-1</td>
<td>&gt;10\textsuperscript{y}</td>
<td>1.27a\textsuperscript{z}</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>B34PC-1</td>
<td>&gt;10</td>
<td>1.22a</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>B34PH-1</td>
<td>&gt;10</td>
<td>2.01a</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>30PC-1</td>
<td>7.76</td>
<td>0.34a</td>
<td>8.17</td>
<td>7.27</td>
</tr>
<tr>
<td>KHSITD</td>
<td>&gt;10</td>
<td>0.76a</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>KH1-2</td>
<td>&gt;10</td>
<td>1.42a</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>KH3-1</td>
<td>&gt;10</td>
<td>1.32a</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

\textsuperscript{x} Data average across three experiments.

\textsuperscript{y} EC\textsubscript{50} values were determined by a mycelial growth inhibition assay.

\textsuperscript{z} Values followed by the same letter within a column for the \textit{R. zeae} isolates are not significantly different according to Waller-Duncan $k$-ratio $t$-test ($k=100$).
Table 3.6. EC\textsubscript{50} value of DMI and QoI + DMI fungicides for \textit{R. zeae} isolates collected in 2013.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Difenoconazole</th>
<th>Triadimefon</th>
<th>Triticonazole</th>
<th>Propiconazole</th>
<th>Tebuconazole</th>
<th>Azoxystrobin +</th>
<th>QoI+DMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>B34PA-1</td>
<td>0.04\textsuperscript{a}</td>
<td>4.67</td>
<td>0.52</td>
<td>0.09</td>
<td>0.02</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>B34PC-1</td>
<td>0.02</td>
<td>3.68</td>
<td>0.45</td>
<td>0.10</td>
<td>0.02</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>B34PH-1</td>
<td>0.06</td>
<td>4.72</td>
<td>0.93</td>
<td>0.10</td>
<td>0.02</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>30PC-1</td>
<td>0.04</td>
<td>0.54</td>
<td>0.30</td>
<td>0.09</td>
<td>0.01</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>KHSITD</td>
<td>0.05</td>
<td>5.15</td>
<td>0.68</td>
<td>0.08</td>
<td>0.03</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>KH1-2</td>
<td>0.05</td>
<td>4.16</td>
<td>0.45</td>
<td>0.09</td>
<td>0.02</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>KH3-1</td>
<td>0.05</td>
<td>4.91</td>
<td>0.43</td>
<td>0.09</td>
<td>0.02</td>
<td>0.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>

\textsuperscript{x} Data average across three experiments.
\textsuperscript{y} Values followed by the same letter within a column for the \textit{R. zeae} isolates are not significantly different according to Waller-Duncan \textit{k}-ratio \textit{t}-test (\textit{k}=100).
\textsuperscript{z} EC\textsubscript{50} values were determined by a mycelial growth inhibition assay.
Table 3.7. EC$_{50}$ value of SDHI, chloronitrile, dicarboximide, and QoI + SDHI fungicides for isolates collected in 2013.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Flutolanil</th>
<th>Penthiopyrad</th>
<th>Fluxapyroxad</th>
<th>Chlorothalonil</th>
<th>Iprodione</th>
<th>Pyraclostrobin + Fluxapyroxad</th>
</tr>
</thead>
<tbody>
<tr>
<td>B34PA-1</td>
<td>9.07a$^{xyz}$</td>
<td>1.28a</td>
<td>0.74a</td>
<td>4.90a</td>
<td>5.62a</td>
<td>0.06ab</td>
</tr>
<tr>
<td>B34PC-1</td>
<td>&gt;10</td>
<td>1.94a</td>
<td>0.33a</td>
<td>5.19a</td>
<td>5.37a</td>
<td>0.07ab</td>
</tr>
<tr>
<td>B34PH-1</td>
<td>&gt;10</td>
<td>0.86a</td>
<td>1.26a</td>
<td>6.38a</td>
<td>4.81a</td>
<td>0.10a</td>
</tr>
<tr>
<td>30PC-1</td>
<td>1.01b</td>
<td>0.07a</td>
<td>0.07a</td>
<td>6.01a</td>
<td>2.79a</td>
<td>0.01b</td>
</tr>
<tr>
<td>KHSITD</td>
<td>7.17a</td>
<td>0.70a</td>
<td>0.39a</td>
<td>6.35a</td>
<td>5.10a</td>
<td>0.04ab</td>
</tr>
<tr>
<td>KH1-2</td>
<td>&gt;10</td>
<td>0.43a</td>
<td>0.38a</td>
<td>5.29a</td>
<td>5.34a</td>
<td>0.05ab</td>
</tr>
<tr>
<td>KH3-1</td>
<td>9.94a</td>
<td>0.42a</td>
<td>0.35a</td>
<td>4.47a</td>
<td>5.41a</td>
<td>0.05ab</td>
</tr>
</tbody>
</table>

$^{x}$ Data average across three experiments.

$^{y}$ Values followed by the same letter within a column for the *R. zeae* isolates are not significantly different according to Waller-Duncan $k$-ratio $t$-test ($k$=100).

$^{z}$ EC$_{50}$ values were determined by a mycelial growth inhibition assay.
Fig. 3.2. Comparison in sensitivity of seven *R. zeae* isolates to 16 fungicides *in vitro* in first experiment. Values represent the mean of three replicate plates per concentration per experiment. Bars followed by the same letter are not significantly different according to Waller-Duncan $k$-ratio $t$-test ($k=100$).
Fig. 3.3. Comparison in sensitivity of seven *R. zeae* isolates to 16 fungicides *in vitro* in second experiment. Values represent the mean of three replicate plates per concentration per experiment. Bars followed by the same letter are not significantly different according to Waller-Duncan *k*-ratio *t*-test (*k*=100).
Fig. 3.4. Comparison in sensitivity of seven *R. zeae* isolates to 16 fungicides *in vitro* in third experiment. Values represent the mean of three replicate plates per concentration per experiment. Bars followed by the same letter are not significantly different according to Waller-Duncan *k*-ratio *t*-test (*k*=100).
Fig. 3.5. Comparison in sensitivity of 13 isolates of *R. zeae* to five fungicides *in vitro* in two experiments. Values represent the mean of three replicate plates per concentration per experiment. Bars followed by the same letter are not significantly different according to Waller-Duncan *k*-ratio *t*-test (*k*=100).
Fig. 3.6. Turf quality for plots treated with nine fungicides and control in 2014. Turf quality was visually estimated on a 1 to 9 scale where 1 = bare ground, 6 = acceptable, 9 = best quality.
Fig. 3.7 Area under the disease progress curve (AUDPC) for plots treated with 9 fungicides and control in 2014. AUDPC values were calculated from disease severity data collected 4 times every 3 weeks.
Fig. 3.8. Turf quality in response to curative fungicides applications in 2015. Turf quality was visually estimated on a 1 to 9 scale where 1 = bare ground, 6 = acceptable, 9 = best quality. Bars followed by the same letter are not significantly different according to Fisher’s Protected LSD.