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Non-target effects of strobilurin fungicide applications on creeping bentgrass putting greens during summer stress

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To the Graduate Council:

I am submitting herewith a thesis written by Jesse J. Benelli entitled "Non-target effects of strobilurin fungicide applications on creeping bentgrass putting greens during summer stress." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

Brandon J. Horvath, Major Professor

We have read this thesis and recommend its acceptance:

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(Original signatures are on file with official student records.)

Non-target effects of strobilurin fungicide applications on creeping bentgrass putting greens during summer stress

A Thesis Presented for the
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Degree
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Jesse J Benelli
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DEDICATION

I dedicate this thesis to all my family and close friends. Your encouragement, support, and sacrifice from all of you are surely appreciated.

"*Withholding non-significant results from publication introduces a serious bias in the biological literature and hence has a retrogressive effect on scientific development.*"

- Ryan D. Csada

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I would like to thank my fellow graduate students Matt Elmore, David Shell, Lucas Freshour, Pat Jones, Keith Bartley, Adam Thoms, and Sophy Hu for their friendship and memories. Together we have given new meaning to the phrase 'start early end early'. I would also like to acknowledge fellow turf colleague Matt Hollan. Sometimes I think I can still hear those urea prills.

I also wish to express my gratitude towards Mother Nature. Your entity has allowed me to witness some of the most damaging outbreaks of disease I have ever seen during my time at Tennessee. Mornings after evening thunderstorms were like waking up on Christmas and seeing all the splendors that wait. Some very sick individuals grow perennial ryegrass and colonial bentgrass in TN.

ABSTRACT

Previous research has indicated that strobilurin fungicide applications may improve summer stress tolerance of creeping bentgrass (*Agrostis stolonifera* L.) putting greens. In this experiment, strobilurin fungicides were integrated within a summer fungicide program to evaluate disease severity and plant physiological effects. Fungicide programs were applied on a 'Dominant Southern' creeping bentgrass putting green; all of which consisted of five fungicide spray applications from June to August during 2011 and 2012. The 2nd and 5th application in each program consisted of a strobilurin fungicide. Strobilurin fungicides evaluated included pyraclostrobin, azoxystrobin, fluoxastrobin, or trifloxystrobin. The remaining fungicide applications were identical across programs. Measurements of visual quality, spectral reflectance, turfgrass cover, rooting characteristics, and disease severity were collected. The non-treated control was similar to treated plots in all parameters in 2011, and until 42 and 56 days after initial treatment (DAIT) the following year. At this time, fungicide programs began to exhibit greater visual quality, turfgrass cover, and spectral reflectance compared to the non-treated control. Differences in physiological effects coincided with the first observation of brown patch (*Rhizoctonia Solani* Kühn) in the experimental area. These data suggest that strobilurin fungicides exhibit excellent efficacy for disease control during summer, and that direct physiological effects may be transient and observable over the long term.

A 2-year field experiment was conducted to determine if applications of pyraclostrobin, azoxystrobin, fluoxastrobin, and trifloxystrobin could improve plant physiological effects of a 'Dominant Southern' creeping bentgrass putting green in the absence of visible foliar disease. Experimental units were arranged in a split-plot randomized complete block design. A fungicide mixture containing chlorothalonil and iprodione and a no fungicide treatment served as the whole-plots. Chlorothalonil and iprodione were applied at 14-day intervals. Sub-plots were treated with strobilurin fungicides (azoxystrobin, pyraclostrobin, fluoxastrobin, and trifloxystrobin) and a non-treated control. Sub-plots were applied at 14-day intervals. Neither differences in disease severity nor any physiological effect was observed on any assessment date over the course of two years. The results of this experiment suggest that while strobilurin fungicides perform well for disease control, detectable plant physiological effects under field conditions were limited.

PREFACE

PRELIMINARY RESEARCH

Field Trial

A preliminary field trial was conducted in 2011 on a ‘Penn A-1’ creeping bentgrass (*Agrostis stolonifera* L.) putting green located at the Pee Dee Research and Education Center (Florence, SC). Research was conducted to evaluate plant health effects of strobilurin based fungicide programs under severe disease pressure. The putting green was established according to USGA (United States Golf Association, 2007) specifications. Mowing was performed five times per week at 3 mm. Overhead irrigation was applied as needed to prevent. The putting green received 5 kg ha^{-1} N from urea every two weeks throughout the trial period.

Treatments were arranged in a randomized complete block design with four replications. Plots measured 0.9 x 3.0 m. Fungicide programs were initiated on 17-May and subsequent applications were made on 14-day intervals (Table 1). Strobilurin treatments were applied at the 2^{nd} and 5^{th} application interval and included 1) azoxystrobin (Heritage TL; Syngenta Crop Protection, Greensboro, NC) at 0.61 kg ai ha^{-1}; 2) pyraclostrobin (Insignia SC; BASF Corporation, Research Triangle Park, NC) at 0.55 kg ai ha^{-1}; and 3) fluoxastrobin (Disarm; Arysta LifeScience North America LLC, Cary, NC) at 0.55 kg ai ha^{-1}; and 4) A non-treated control receiving no fungicide treatments was included for comparison. A breakdown of each fungicide program is presented in Table 1. Treatments were applied as a foliar spray using two flat fan nozzles (TeeJet 8004EVS; Spraying Systems Co., Roswell, GA) calibrated to deliver a spray volume of 847 L per ha^{-1}.

Data Collection

Visual turfgrass quality, spectral reflectance, and disease severity were assessed throughout the trial period. Visual turfgrass quality was measured on a 1 to 9 scale where a rating of 1 was considered bare soil and a rating of 9 was considered exceptional. A rating of 6 was considered acceptable. Spectral reflectance was measured using a mobile spectrophotometer (ASC-210; Holland Scientific, Lincoln, NE) and configured similarly to Jiang et al. (2009). This instrument has a built-in light sensor that simultaneously emits visible and near infrared light onto the turf canopy. The sensor was fitted onto a golf push cart 0.75 m above the canopy and pushed across the center of each plot to obtain normalized difference vegetation index (NDVI) and ratio vegetation index (RVI). Disease severity was evaluated whenever symptoms were present within the trial area. Brown patch (*Rhizoctonia solani* Kühn) was assessed by visually estimating the percent turf area infected. Dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) was assessed by counting the number of dollar spot foci per plot.

Two weeks after each strobilurin application an analysis of percent turfgrass cover was quantified using digital image analysis (DIA). Digital images were captured using a Canon G12 (Canon G12, Canon Inc., Japan) digital camera. The camera was mounted inside a 0.28 m^2 box equipped with four fluorescent light bulbs. Image size in this experiment was 307,200 pixels. Images were analyzed similar to the methods and configuration from Richardson et al. (2001) using SigmaScan Pro software (Version 5.0; SPSS Inc., Chicago, IL). Pixels classified as green turf expressed a hue range of 45^o to 120^o and saturation defined between 0% and 100%.

A root morphology assessment was conducted at two weeks after the 2^{nd} strobilurin application. A soil profiler (Soil profiler, Forestry Suppliers Inc., Jackson, MS) was used to extract 14 soil profiles from each plot. Soil profiles measured 25 cm^2 and were pulled from a depth of 18 cm into the rootzone. Soil profiles were gently rinsed with water to remove sand and organic matter. The remaining roots were scanned and analyzed for total root length, root surface area, root diameter and root volume using the WinRhizo Pro system (WinRhizo Pro, Regent Instruments Inc., Canada). Procedures were similar to those outlined in Beasley and Branham (2007) and Brosnan et al. (2010).

Data were subjected to analysis of variance (ANOVA) using PROC MIXED with code generated by the DANDA macro (Saxton, 2010) in SAS (Statistical Analysis Software, Inc., Cary, NC). Means were separated using Fisher's protected least significant difference (LSD) at $\alpha = 0.05$. A combined ANOVA was conducted to test for significant interactions between experimental runs. The area under the progress curve (AU(x)PC) was calculated for each of the evaluation parameters where (x) is a parameter of interest. The (AU(x)PC) describes the course of increase or decrease in (x) parameter over time (Campbell and Madden, 1991).

Results

Analysis of variance revealed significant differences in visual turfgrass quality, spectral reflectance, and disease severity. No significant differences in percent turfgrass cover or rooting characteristics were observed (data not presented).

All strobilurin programs resulted in acceptable quality on every evaluation date. Unacceptable quality was observed within the non-treated control on 6 of the last 8 rating dates (data not presented). Analysis of area under the visual quality progress curve (AUVQPC) revealed significant differences among treatments (Figure 1). All strobilurin fungicide programs exhibited greater AUVQPC than the non-treated control. Fungicide programs featuring pyraclostrobin had significant higher AUVQPC than the fluoxastrobin programs, but pyraclostrobin programs were not statistically different from azoxystrobin programs (Figure 1).

Spectral reflectance gradually declined in all treatments throughout trial period. Strobilurin programs had significantly greater spectral reflectance than the non-treated control beginning 21 days after initial treatment (DAIT). Pyraclostrobin programs resulted in significantly greater NDVI than azoxystrobin and fluoxastrobin programs from 56 to 84 DAIT. Azoxystrobin and fluoxastrobin programs were not significantly different on any evaluation date (Table 2). All fungicide programs had significantly greater area under the NDVI progress curve (AUNDVIPC) and area under the RVI progress curve (AURVIC) compared to the non-treated control. Pyraclostrobin programs exhibited higher AUNDVIPC and AURVIC than both azoxystrobin and fluoxastrobin programs. No statistical differences were reported for spectral reflectance measurements between azoxystrobin and fluoxastrobin programs (Figures 2, 3).

Environmental conditions were conducive for brown patch development during this experiment. Peak brown patch activity occurred at 56 DAIT. On this date, the non-treated control exceeded 40% disease severity. Pyraclostrobin programs had trace amounts of brown patch on this date (< 2%) and were significantly lower than programs with either azoxystrobin or fluoxastrobin which each had 9% brown patch, respectively (data not presented). An analysis of the area under the brown patch progress curve

(AUBPPC) revealed significant differences among treatments. Pyraclostrobin programs had significantly lower AUBPPC than all other treatments. Programs featuring azoxystrobin and fluoxastrobin were statistically similar in this assessment and each had significant lower AUBPPC than the non-treated control (Figure 4).

The aforementioned differences in disease severity may contribute to field observable differences in plant physiological effects. If creeping bentgrass plants are subjected to increased disease pressure then the plant's physiology could be deteriorated enough to cause a field observable degradation of turf quality. A regression analysis revealed a strong correlation ($R^2 = 0.82$) between disease severity and spectral reflectance measurements such as NDVI (Figure 5).

Conclusion

All strobilurin programs maintained acceptable turf quality and disease control throughout the experiment. Pyraclostrobin programs had greater AUNDVIPC and AURVIPC measurements than all other strobilurin programs. Pyraclostrobin programs also exhibited a greater AUVQPC compared to fluoxastrobin treated programs; however pyraclostrobin and azoxystrobin programs were not statistically different. Fluoxastrobin and azoxystrobin programs were nearly identical in the AUVQPC, AURVIPC, and AUNDVIPC analyses.

Differences in these physiological effects between strobilurin programs could be due to a number of factors. Stressors imposed by the environment could adversely affect creeping bentgrass plants differently when exposed to different strobilurin fungicides. Another potential explanation is differences in disease control efficacy between strobilurin treatments. However, it remains impractical to discern whether differences in physiological effects are likely the result of environmental stressors, disease control efficacy, or other unknown factors from data collected at a single location in one growing season. It is anticipated that additional data will become available to replicate this trial over time.

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Table 1. Summer fungicide program integrated with strobilurin fungicides.

Application interval[†]	Fungicide[‡]	Fungicide class	Rate (kg ha^{-1})
1,7,8	Iprodione	Dicarboximide	3.05
2,5	Strobilurin[§]	Quinone outside inhibitor	
3	Thiophanate-methyl	Benzimidazole	3.16
	Chlorothalonil	Benzonitrile	12.65
4,6	Fosetyl Aluminum	Phosphonate	9.76
	Chlorothalonil	Benzonitrile	8.05

† Applications were initiated on 17-MAY in 2011. Subsequent applications were made on 14-day intervals.

‡ Fungicides were applied using two, flat-fan, nozzles calibrated to deliver a spray volume of 807 L per ha^{-1}.

§ Azoxystrobin, Pyraclostrobin, and Fluoxastrobin were applied at rates 0.55, 0.61, and 0.55, kg ha^{-1}, respectively, on the 2^{nd} and 5^{th} application interval.

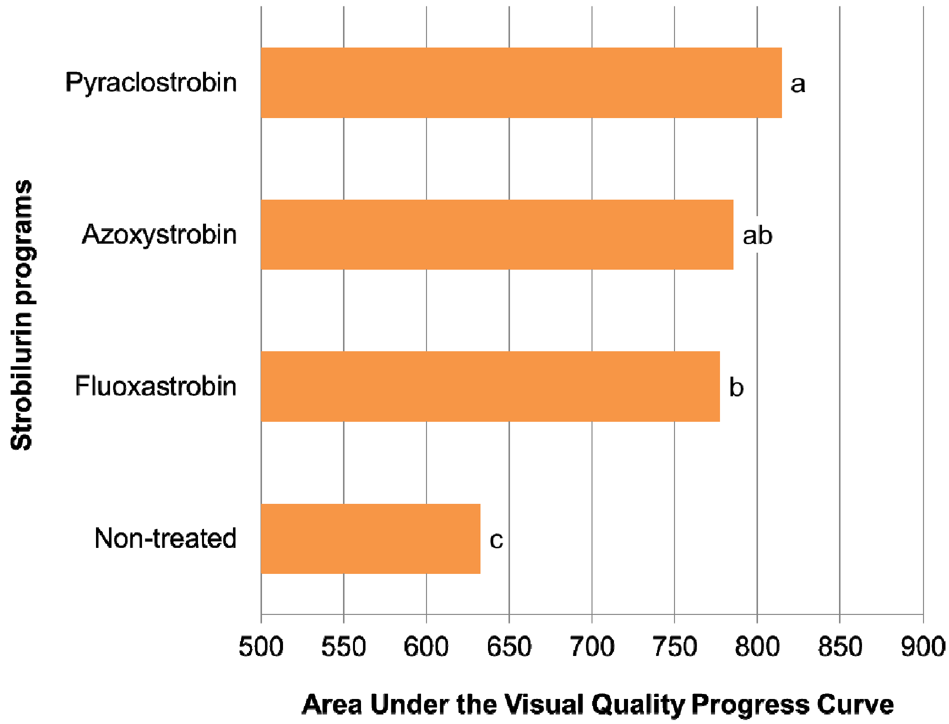

Figure 1. Area under the visual quality progress curve values (AUVQPC) in response to strobilurin programs in Florence, SC. AUVQPC were calculated from visual turfgrass quality data collected for 13 rating dates. Bars followed by the same letter are not significantly different according to Fisher's protected least significant difference.

Table 2. Spectral reflectance measurements for normalized difference vegetation index (NDVI) in response to strobilurin fungicide programs. Fungicide programs were located in Florence, SC, on a 'Penn A-1' creeping bentgrass (*Agrostis stolonifera* L.) putting green.

Fungicide Program[†]	NDVI				
	Days after initial treatment				
	56	63	71	79	84
Pyraclostrobin[‡]	.7520	.7560	.7865	.6985	.6985
Azoxystrobin	.7397	.7427	.7585	.6743	.6723
Fluoxastrobin	.7357	.7368	.7583	.6650	.6643
Non-treated	.7020	.7113	.7278	.6255	.6308
$LSD_{0.05}$	.0121	.0065	.0124	.0187	.0175

† Applications were initiated in on 17-May in 2011. Subsequent applications were made on 14-day intervals.

‡ Pyraclostrobin, azoxystrobin, and fluoxastrobin were applied at rates 0.61 0.55, and 0.55 kg ha^{-1}, respectively, on 14 and 56 days after initial treatment.

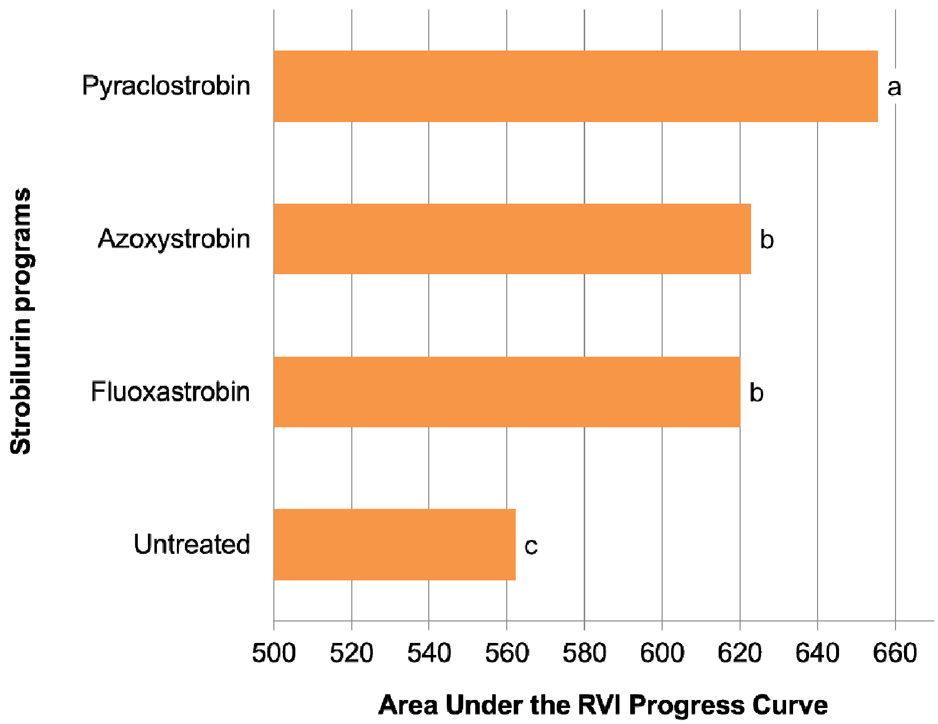

Figure 2. Area under the ratio vegetation index progress curve (AURVIPC) in response to strobilurin programs in Florence, SC. AURVIPC were calculated from 13 rating dates. Bars followed by the same letter are not significantly different according to Fisher's protected least significant difference.

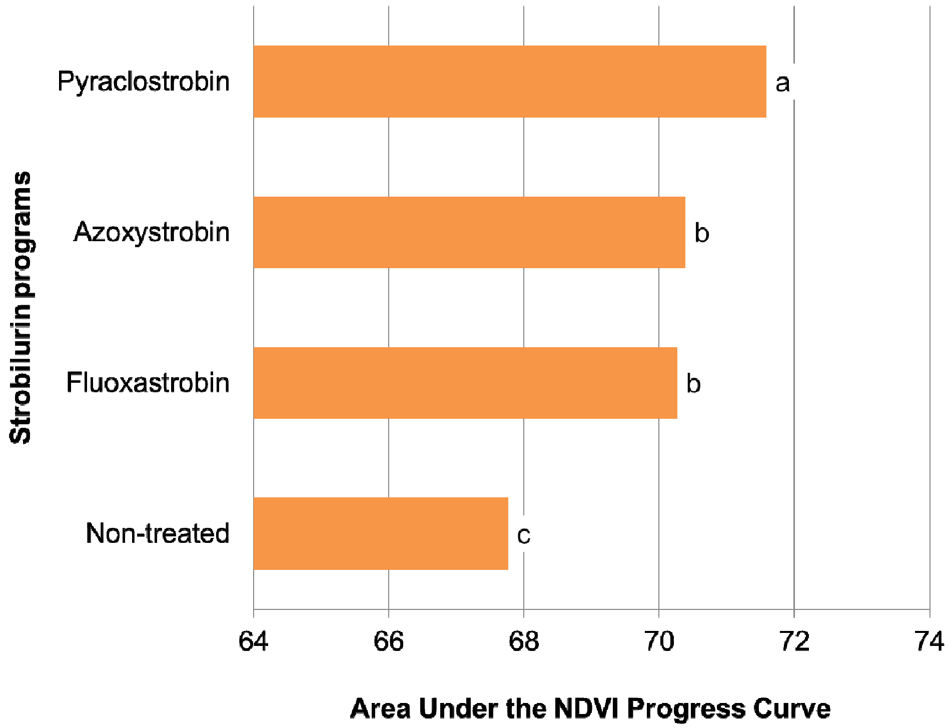

Figure 3. Area under the normalized difference vegetation index progress curve (AUNDVIPC) in response to strobilurin programs in Florence, SC. AUNDVIPC were calculated from 13 rating dates. Bars followed by the same letter are not significantly different according to Fisher's protected least significant difference.

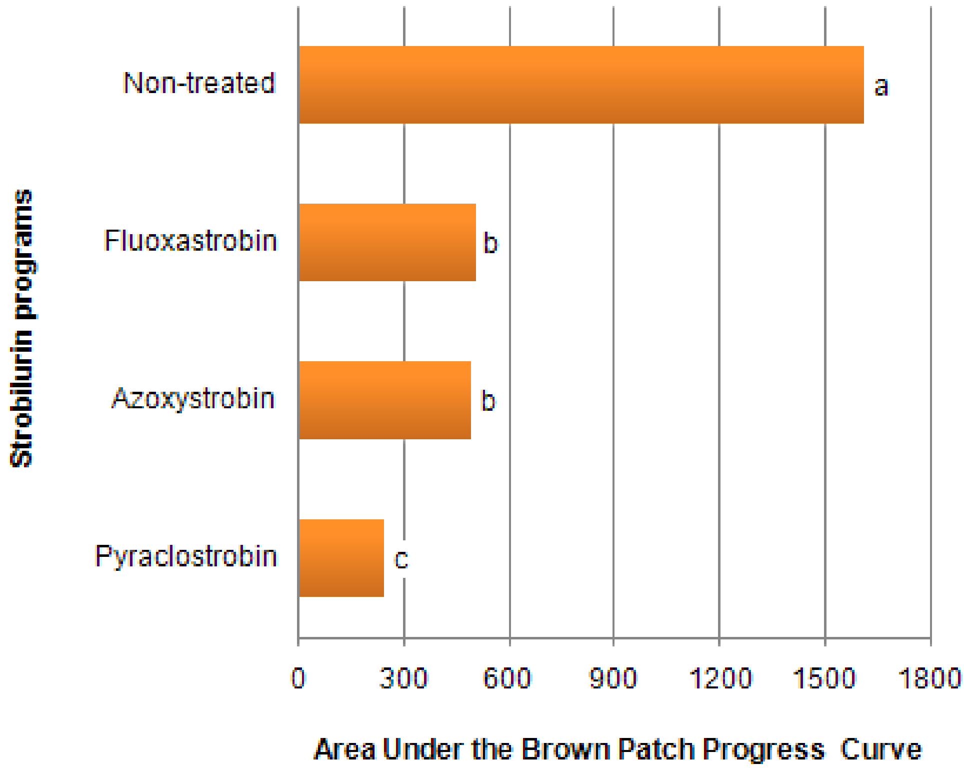

Figure 4. Area under the brown patch progress curve (AUBPPC) in response to strobilurin fungicide programs in Florence, SC. AUBPPC values were calculated from disease severity data collected from 11 rating dates. Bars followed by the same letter are not significantly different according to Fisher's protected least significant difference.

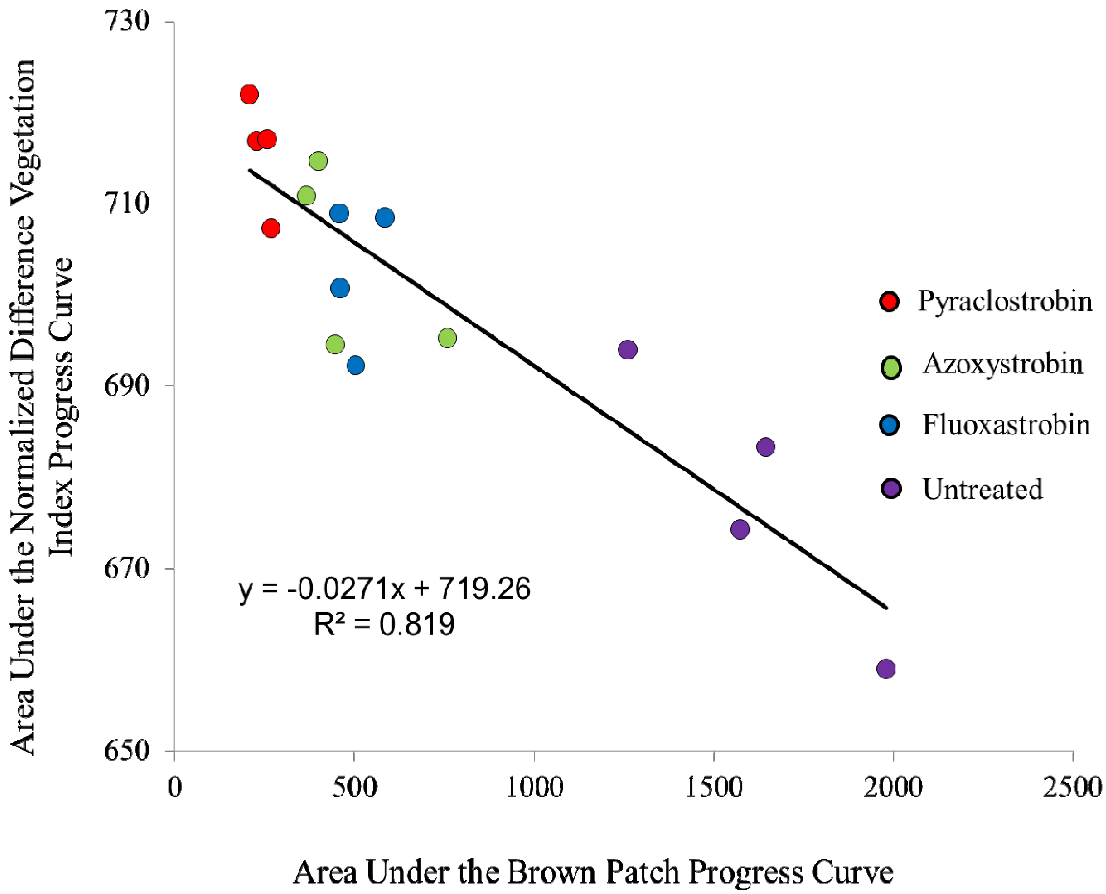

Figure 5. Linear regression analysis of the AUNDVIPC and the AUBPPC comparing the season-long effect of disease on the spectroscopic response of creeping bentgrass.

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CHAPTER I
LITERATURE REVIEW

CREEPING BENTGRASS

Creeping bentgrass (*Agrostis stolonifera* L.) is a perennial C_3 grass belonging to Family Poaceae, Subfamily Pooideae, and Tribe Avenae (Beard, 2002). This species is predominantly established in turfgrass systems through seed and spreads laterally across the soil surface through stoloniferous growth (Beard, 2002). The primary use of creeping bentgrass in the United States is for golf course surfaces due to its fine texture, dense growth habit, and tolerance to frequent close mowing (Turgeon, 1999). This adaptability to tolerate mowing heights at less than 3 mm is the predominant reason creeping bentgrass is the most seeded turfgrass for golf course putting greens throughout the temperate regions of the United States (Lyman et al., 2007).

As a cool season turfgrass, creeping bentgrass is seldom used in the southern most states due to its poor tolerance to prolonged heat. In these regions, hybrid bermudagrass (*Cynodon dactylon* L. × *C. transvaalensis* Burtt-Davy) greens are more commonly established (Lyman et al., 2007). The difficult management decision lies in selecting the proper grass in areas that experience high temperatures in summer and freezing conditions during winter. These areas of the country are often referred to as the transition zone. The transition zone is an area between warm and cool regions where the quality of both C_4 and C_3 grasses declines due to environmental conditions. In the transition zone, creeping bentgrass can maintain its green color throughout winter and continues to provide exceptional quality during spring and fall months (Dunn and Diesburg, 2004). The quality and health of creeping bentgrass is most compromised when prolonged heat sets in during summer (Dernoeden, 2002).

Creeping bentgrass is susceptible to a number of abiotic and biotic stressors during summer months. The gradual deterioration of creeping bentgrass quality through periods of summer stress is referred to as Summer Bentgrass Decline (SBD) (Lucas, 1996). SBD is an environmentally induced condition that is attributed to supraoptimal air and soil temperatures. With exposure to air and soil temperatures of 35ºC root and shoot growth, photosynthetic rate, and canopy density of creeping bentgrass is reduced (Xu and Huang, 2000, 2001). Creeping bentgrass stress sensitivity to temperature extremes is often exacerbated by water deficit or soil saturated conditions (Dernoeden, 2002). During water deficit stress, evapotranspiration is restricted when leaf tips curl and stomates close (Fry and Huang, 2004). When evapotranspiration is reduced, the plant loses its ability to take up soil applied nutrients and pesticides. Saturated rootzones create an anaerobic environment that restricts oxygen movement through the plant (Turgeon, 1999). During this condition, transpiration stops and the plant cannot cool themselves properly during periods of high temperatures (Fry and Huang, 2004). Creeping bentgrass managed under water deficit or excessive moisture conditions can predispose the plant to pathogen activity (Burpee and Martin, 1992).

DISEASE MANAGEMENT FOR CREEPING BENTGRASS

Creeping bentgrass is susceptible to a wide variety of diseases during summer months. Copper spot (*Gloeocercospora sorghi* Bain and Edgerton), red leaf spot (*Dreshslera erythrospila* Shoemaker), and particularly dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) are active during milder environmental conditions (Smiley et al., 1992). In the transition zone, these diseases predominantly occur during late spring and early fall months (Dernoeden, 1989). During warmer climatic conditions, brown patch (*Rhizoctonia solani* Kühn) causes significant leaf blighting of creeping bentgrass turf (Burpee and Martin, 1992). Creeping bentgrass is also prone to opportunistic diseases that take advantage of weakened or senescing plant tissues. Disease occurrence of *Leptosphaerulina* leaf blight (*Leptosphaerulina trifoli* McAlpine), *Curvularia* diseases (*Curvularia* spp.), *Pythium* root diseases (*Pythium* spp.), or anthracnose (*Colletotrichum cereale* Manns sensu lato Crouch, Clarke, and Hillman) can be an indication that creeping bentgrass is under stress (Inguagiato et al., 2008; Mitkowski and Browning, 2004; Falloon, 1975).

To combat disease, turfgrass managers incorporate an integrated pest management approach that includes cultural and chemical programs. Sand topdressing, spoon-feeding nitrogen, dew removal, and irrigation management are among the most documented cultural control methods used to manage several turfgrass diseases (Jiang et al., 1998; Fidanza and Dernoeden, 1996; Inquagiato et al., 2009). However, despite these practices, disease severity may occur when environmental conditions remain conducive for disease development (McDonald et al., 2006; Delvalle et al., 2011). Chemical control becomes essential when environmental conditions hinder the growth of the plant but promote rapid disease development.

Among the first disease control products in the early 20th century were preparations of a copper sulfate and hydrated lime mixture, known as the Bordeaux mixture (Hawkings, 1912). Chlorophenol mercury, cadmium succinate, and the production of synthetic organic fungicides soon followed in the 1940's (Godfrey, 1925;

Jackson, 1964; Harrington, 1942). However, most of these products had high use rates, required frequent application intervals, targeted few diseases, and were often phytotoxic to desirable plant species, and were frequently highly toxic to humans (Latin, 2011). The advent of newer compounds in the 1960's (chlorothalonil, mancozeb, and thiophanate-methyl) and 1970's (iprodione, vinclozolin, and triadimefon) made broad spectrum disease control more efficient with a reduced amount of potential harm to the applicator (Latin, 2011). By 1990, demethylation inhibitors (DMI), benzimidazoles, dicarboximides, and multi-site compounds were the predominant modes of action used in turfgrass landscapes (Couch, 1995). In the mid 1990's a new broad spectrum class of fungicides, the strobilurins, was introduced for disease control.

Strobilurin fungicides, sometimes referred to as quinone outside inhibitors (QoI), [Fungicide Resistance Action Committee (FRAC) Code 11] were first registered for turfgrass use with the introduction of azoxystrobin (Heritage WDG; Syngenta Crop Protection, Greensboro, NC) in 1996. Competing chemical manufacturers soon introduced their own strobilurin fungicide. As of 2012, the list of strobilurin fungicides has broadened to include trifloxystrobin (Compass: Bayer Environmental Science, Research Triangle Park, NC), pyraclostrobin (Insignia WG; BASF Corporation, Research Triangle Park, NC), and fluoxastrobin (Disarm 480 SC; Arysta LifeScience North America LLC, Cary, NC). These control products were discovered in part from a natural wood rotting basidiomycete fungus, *Strobilurus tenacellus,* which produces the fungicidal derivative methoxyacrylic acid (Bartlett et al., 2001). Strobilurin fungicides have been chemically modified to become less prone to photo degradation and are classified as reduced risk pesticides by the Environmental Production Agency (EPA) (Köhle et al., 2002).

Strobilurin fungicides inhibit mitochondrial respiration in fungi by binding at the ubiquinone (Qo) site located within the cytochrome bc_1 complex of the mitochondrial membrane (Bartlett et al., 2001). The result is the inactivation of the ubiquinol oxidase enzyme that produces a blockage of electron transport inhibiting the production of ATP, the main source of energy needed for fungal growth (Bartlett et al., 2001). Strobilurin fungicides are broad spectrum in their ability to control a variety of basidiomycete, ascomycete, and oomycete plant pathogens (Wong et al., 2006). As of 2009, strobilurin fungicides account for over 2.6 billion dollars in global sales annually, representing 22% of the fungicide marketplace (Kramer et al., 2012). Their popularity is due in part to their aforementioned broad-spectrum capabilities, and because of their curative ability against existing infection. These fungicides all penetrate through the leaf surface; however they vary in the ability to be redistributed throughout the plant. Azoxystrobin and fluoxastrobin are acropetal penetrants that move through the xylem and toward the leaf tips (Häuser-Hahn et al., 2004). Pyraclostrobin and trifloxystrobin are considered localized penetrants as they move throughout the leaf by translaminar movement (Stierl et al., 2002).

Strobilurin fungicides are particularly effective against *Rhizoctonia* diseases such as brown patch. Efficacy against this disease using strobilurin fungicides is well documented (Uddin et al., 2010; Soika and Tredway, 2010). Other diseases of creeping bentgrass controlled, or suppressed by strobilurin fungicides include anthracnose, fairy ring, and *Pythium* root diseases (Wong et al. 2006; Fidanza and Bagwell, 2005; Kerns et al., 2009). Perhaps the greatest disparity among strobilurin fungicides is their ability to control dollar spot. Azoxystrobin and trifloxystrobin have little to no effect on this disease (Latin, 2006). In some instances applications of azoxystrobin have increased dollar spot severity compared to a non-treated control in disease efficacy trials (Vincelli et al., 1996). However, pyraclostrobin and fluoxastrobin have been shown to suppress this disease (Aynardi et al., 2011). The advantages of strobilurin fungicides include their low use rates, extended residual efficacy and reduced toxicity on non-target organisms compared with other classes of fungicides such as benzimidazoles, dicarbamates and dimethylation inhibitors, (Latin, 2011). In addition to their fungistatic properties, some strobilurin fungicides, such as pyraclostrobin, are now labeled to provide plant health benefits in absence of visible disease (Anonymous, 2010).

PHYSIOLOGICAL EFFECTS OF STROBILURIN FUNGICIDES

The bc_1 complex of the mitochondrial membrane is located within all eukaryotes, not just fungi. The basis for these fungicides potentially affecting a plant's physiological processes is that they temporarily disrupt mitochondrial respiration in plants as well as fungi (Bartlett et al., 2001). A partial or transient inhibition of the cytochrome respiratory pathway in plants triggers the alternative oxidase pathway (AOX) (Wagner and Krab, 1995). This allows for an alternative pathway for mitochondrial electron transport away from the ubiquinone pool (Wagner and Krab, 1995). The up-regulation of the AOX pathway can facilitate several stress related plant responses.

Mitochondrial production of reactive oxygen species (ROS) is reduced when the AOX pathway is activated compared to the primary cytochrome pathway (Purvis, 1997). The generation of excessive cellular ROS ($O_2^{\cdot -}$, $^{\cdot}OH$, O_2, and H_2O_2) impairs the ability of the plant to withstand oxidative stress (Halliwell, 2006). Activation of the AOX pathway may shift the balance in favor of anti-oxidants (superoxide dismutases, catalase, peroxidases, glutathione, and ascorbate) and away from harmful ROS during stressful periods. Wu and Tiedemann (2001) demonstrated that strobilurin fungicides significantly reduced physiological leaf spot symptoms on spring and winter barley (*Hordeum vulgare* L.) when exposed to elevated levels of ozone. They found that strobilurin fungicides increase anti-oxidant production in barley plants favoring rapid quenching of ROS. This reduction in ROS may also impact a plant's tolerance to excess light. Under high light intensities, photoinhibition may occur in addition to damaging the photosystem II receptors (PSII) causing rapid generation of ROS and reduced net photosynthesis (Zhang et al., 2011). Activation of the AOX pathway inhibits this over production of ROS resulting in reduced cellular damage under high light intensity (Zhang et al., 2011).

Strobilurin fungicides may also indirectly enhance nitrogen assimilation in higher plants (Köhle et al., 2002). The partial or transient inhibition of the cytochrome pathway activates nitrate reductase (NR) due to the rapid reduction of cellular adenosine triphosphate (ATP). Glaab and Kaiser (1999) observed that a buffer solution of the strobilurin kresoxim-methyl (KROM) prevented the inactivation of NR in spinach (*Spinacia oleracea* L.) leaf discs while leaf discs without KROM had a 20% decrease in NR. Furthermore, if NR is activated by KROM, an accumulation of nitrite in leaf tissue is observed. This increase in nitrites via NR produces the byproduct nitric oxide (NO)

(Yamasaki, 1999). This signaling molecule in plants has been suggested to cause a cascade of secondary reactions. NO stimulates the plant's self-defense system against pathogen attack by signaling cyclic ribose messengers (Bolwell, 1999). In addition, NO accumulation in plants can cause the inhibition of 1-aminocyclopropane-1-carboxylate (ACC) synthase (Leshem, 2000). This enzyme catalyzes the first step of ethylene biosynthesis.

Ethylene is a plant hormone that is commonly associated with fruit ripening and plant senescence. During stages of active growth, ethylene production can trigger a rapid accumulation of ROS, thus reducing the plant's ability to cope with environmental stressors (Gortz et al., 2008). Strobilurin fungicides causing a partial inhibition of ethylene may cause a "greening" effect observed in certain agricultural crops. Grossmann and Retzlaff (1997) demonstrated that wheat (*Triticum aestivum* L.) plants treated with KROM had a 50% reduction in ethylene formation compared to non-treated wheat plants. The authors proposed it was this reduction of ethylene coupled with an increase in cytokinins that resulted in a greening response. A delay in leaf senescence may also lengthen the grain filling period of several crops including corn (*Zea mays* L.) and wheat (Ammermann et al., 2000; Godwin et al., 2000; Bryson, 2000) resulting in potential yield increases. This increase in yield has been suggested to be the result of prolonged photosynthetic ability in the lower (older) leaves as a result of delayed senescence (Grossman et al., 1999). However, other studies suggest this increase in grain yield after strobilurin fungicide applications is much less pronounced in low disease pressure environments (Swoboda and Pedersen, 2009; Weisz et al., 2011).

Strobilurin fungicides altering physiological responses within the aboveground portion of the plant may also have effects on root growth and development. A seed dressing containing fluoxastrobin did not alter shoot or leaf formation of barley, but slightly stimulated root growth in varying environmental conditions (Gortz et al., 2008). Brosnan et al. (2010) observed that pyraclostrobin increased rooting in ‘Penn A-1’ creeping bentgrass. Conversely, azoxystrobin decreased rooting compared to the non-treated control in a greenhouse environment, but this effect was not detected on ‘Penncross’ creeping bentgrass. It is unclear why strobilurin fungicides may increase rooting in plants. Brosnan et al. (2010) suggested that these strobilurin treated turfgrass plants displayed a response similar to what would be expected following treatment with low doses of auxin, a plant growth hormone known to promote adventitious rooting (Grossman and Retzlaff, 1997; Taiz and Zeiger, 1998; Köhle et al., 2002).

MEASURING PHYSIOLOGICAL EFFECTS

VISUAL TURFGRASS QUALITY

Visual turfgrass quality is a qualitative measurement used to evaluate the overall health and performance characteristics of turfgrass. This subjective measurement is an integrated value including color, density, uniformity, growth, and leaf texture (Beard, 1973). The evaluator will take into consideration these qualities and factor in the purpose of the selected turfgrass site. Evaluating turfgrass quality in a lawn or roadside setting will be qualitatively different compared to a assessing a creeping bentgrass putting green.

Turfgrass quality is typically measured on a 1 to 9 scale where 1 = bare soil and 9 = exceptional quality. A quality rating of 6 has become a standard for acceptable quality (National Turfgrass Evaluation Program, 2013).

Visual quality has been an accepted qualitative evaluation for the aesthetics and functionality of turfgrass for at least 75 years (North and Odland, 1934) and continues to be integrated into performance evaluation in modern experiments (Watson et al., 2012). However, there is no widely accepted turfgrass quality formula published in peer-reviewed journals. This is a potential reason why visual turfgrass quality ratings are difficult to duplicate from one study to another and from one university to another (Horst et al., 1984). Another reason why visual ratings are difficult to duplicate is subjective bias among evaluators. Landschoot and Mancino (2000) observed differences among evaluators for visual color, lightness, and chroma assessments on creeping bentgrass. Recently, quantitative measurements have been developed to assess various indicators of turfgrass performance.

NORMALIZED DIFFERENCE VEGETATION INDEX

Normalized Difference Vegetation Index (NDVI) is a quantitative spectral reflectance measurement used to estimate leaf area index and absorbed photosynthetic active radiation of vegetation. It is defined as the near-infrared (NIR) minus visible reflectance divided by NIR plus visible reflectance. NDVI can be broken down into two separate sub-categories. Red reflectance is associated with the amount of chlorophyll adsorption and is visible (Knipling, 1970). It is this component of NDVI which is commonly correlated with visual turfgrass quality ratings (Bell et al., 2004). Near Infrared Reflectance (NIR) is a measure of light scattering within plant cells and is considered to be non-visible. NIR is generally associated with the water status of a plant and the presence of senescing leaf tissue (Penuelas et al., 1993; Jensen, 2007).

NDVI has been used as an indicator for turfgrass quality, cover, and injury among several cultivars of seashore paspalum *(Paspalurn vaginaturn* Swartz) and hybrid bermudagrass (Trenholm, 1999). NDVI as a measure to predict visual quality has been investigated in recent years. Bremer et al. (2011) conducted a 3-year field trial investigating relationships between NDVI and visual ratings on three cultivars of Kentucky bluegrass and one cultivar of tall fescue. The authors observed coefficients of determination correlations (r^2 = 0.38 – 0.83) between NDVI and visual ratings in Kentucky bluegrass and (r^2 = 0.05 – 0.56) in tall fescue. In this study, higher r^2 values were observed during the 2nd year of the trial when the authors noted greater heat and water deficient stress among the plots; thus broadening the range of visual quality.

NDVI was also able to detect increasing nitrogen and chlorophyll content in leaf tissue of ‘SR1020’ creeping bentgrass (Bell et al., 2004). NDVI has been used to assess various abiotic stresses associated with perennial ryegrass (*Lolium perenne* L.) (Jiang et al., 2009). Measurements of NDVI can detect brown patch severity in tall fescue (*Festuca arundinacea* Schreb.) (Green et al., 1998). Anderson and Fermanian (2009) observed that NDVI measurements detected disease infection of brown patch and *Pythium* blight up to 25 h before symptoms or signs were visually present.

DIGITAL IMAGE ANALYSIS

Digital imaging software allows for a rapid estimation of turfgrass characteristics including percent cover, color, disease severity, and other performance characteristics. Digital image analysis (DIA) using Sigma Scan software can estimate percent canopy coverage in soybean (*Glycine max* L.) fields and bermudagrass establishments (Purcell, 2000; Richardson et al., 2001). Karcher and Richardson (2003) established color parameters using hue, saturation, and brightness for creeping bentgrass cultivars to measure color differences in plots after nitrogen fertilization. Digital analysis of green cover can quantify turfgrass recovery following drought stress and winter injury in tall fescue (*Festuca arundinacea* Shreb.), Kentucky bluegrass (*Poa pratensis* L.), and zoysiagrass (*Zoysia* spp.) landscapes (Karcher et al., 2008; Richardson et al., 2008; Patton and Reicher, 2007). DIA can also quantify percent turf blighting from diseases such as dollar spot (Horvath and Vargas, 2005; Ellram et al., 2007).

The use of turfgrass quality to measure turfgrass quality has been investigated by a number of authors. Bunderson et al. (2009) observed a weak correlation ($r^2 = 0.51$) between digital analysis of percent green cover compared with visual turfgrass quality ratings among native grasses. The authors suggested that digital analysis methods should not replace visual quality methods but rather supplement the human aspect of turfgrass quality ratings.

ROOTING ANALYSIS

Supraoptimal air and soil temperatures can adversely affect growth rate, color, and total root mass of creeping bentgrass (Beard and Daniel, 1965; Huang and Gao, 2000; Fagerness and Yelverton, 2001). Furthermore, root length has been identified as a possible characteristic of stress tolerance. Bonos and Murphy (1999) observed that summer stress tolerant varieties of Kentucky bluegrass exhibited 65% more rooting at the 30-45 cm soil depth compared to the intolerant varieties. Similarly, Carrow (1996) screened several warm- and cool-season turfgrasses grown under drought and edaphic stress. Turfgrasses that expressed less visual leaf firing and wilt produced 95% greater root length density compared to less tolerant turfgrasses.

Recently, rooting analysis using WinRhizo software has been incorporated into experiments. WinRhizo can estimate total root length (cm), root surface area (cm^2), mean root diameter (mm), root length density (cm cm^3), and root volume (cm^3). Beasley et al. (2007) reported 48% less root length in Kentucky bluegrass after applications of the plant growth regulator trinexapac-ethyl compared to non-treated plants grown in a hydroponic environment. However, Fagerness and Yelverton (2001) reported no adverse effects of creeping bentgrass rooting under field conditions after applications of trinexapac-ethyl when managed as a putting green. Brosnan et al. (2010) observed that pyraclostrobin increased rooting in ‘Penn A-1’ creeping bentgrass. Conversely, azoxystrobin decreased rooting compared to the non-treated control in a greenhouse environment, but this effect was not detected on ‘Penncross’ creeping bentgrass.

Applications of strobilurin fungicides can improve physiological effects of several agronomic crops in absence of visible disease. However, the physiological effects associated with these fungicides on turfgrass have not been carefully examined in the field. Little published data exists where a direct comparison is made between the performance of azoxystrobin, pyraclostrobin, fluoxastrobin, and trifloxystrobin applied during summer stress. The objective of this research is to evaluate physiological effects of creeping bentgrass during summer stress in response to strobilurin fungicide applications.

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CHAPTER II

PHYSIOLOGICAL EFFECTS OF STROBILURIN FUNGICIDE APPLICATIONS INCORPORATED WITHIN A PROGRAMMATIC FUNGICIDE APPROACH ON CREEPING BENTGRASS

The intent of this manuscript is to publish articles in the peer-reviewed literature. This work is based on contributions by Jesse Benelli, Brandon Horvath, Jim Brosnan, and Dean Kopsell:

My primary contributions to this paper include (i) designing and conducting the experiments, (ii) analyzing and interpreting data, (iii) literature review, and (iv) writing the manuscript.

ABSTRACT

Prior research indicates that strobilurin fungicides may mitigate creeping bentgrass (*Agrostis stolonifera* L.) stress through disease control and a promotion of plant physiological effects. A 2-year field experiment was conducted on a 'Southern dominant' creeping bentgrass putting green in Strawberry Plains, TN, to examine disease control efficacy and turf quality following treatment with fungicide programs featuring either azoxystrobin, pyraclostrobin, fluoxastrobin, and trifloxystrobin at rates of 0.55, 0.61, 0.55, and 0.38 kg ha^{-1}, respectively. Programs consisted of five spray applications from June to August at 14-day intervals. Strobilurin fungicides were applied at the 2^{nd} and 5^{th} application interval with all other fungicide applications remaining identical across treatments. Measurements of disease severity, turf quality, spectral reflectance, turf cover, and rooting length were evaluated every two weeks. A rooting analysis was conducted at the termination of the trial period. In 2011, no significant differences were detected regardless of parameter. In 2012, all fungicide programs reduced brown patch (*Rhizoctonia solani* Kühn) severity compared to the non-treated at 42 and 56 days after

initial treatment. Concomitantly, turf quality, turf cover, and spectral reflectance were increased compared to the non-treated control. Programs containing pyraclostrobin and azoxystrobin yielder higher turf cover values than the other strobilurin programs; however no differences in brown patch severity were detected among the strobilurins tested. This work demonstrates that strobilurin fungicides perform well for disease control, but observations of secondary plant health effects were not observed.

INTRODUCTION

Creeping bentgrass (*Agrostis stolonifera* L.) putting greens grown in the transition zone are susceptible to a number of abiotic and biotic stressors during summer months (Dernoeden, 2002). Exposure to supraoptimal air and soil temperatures, ultraviolet light radiation, and moisture stress can cause significant physiological damage to creeping bentgrass (Huang and Gao, 2000; Brennan and Halisky, 1970; Schmidt and Snyder, 1984). In addition, creeping bentgrass putting greens are susceptible to diseases such as brown patch (*Rhizoctonia solani* Kühn), dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett), anthracnose (*Colletotrichum cereale* Manns sensu lato Crouch, Clarke, and Hillman), and *Pythium* spp. (Wong et al., 2006). To combat disease, turfgrass managers incorporate an integrated pest management approach including cultural and chemical programs. Irrigation scheduling, nutrient management and raising mowing heights are cultural practices known to affect disease development and plant vigor (Jiang et al., 1998; Fidanza and Dernoeden 1996; Inquagiato et al., 2009). However, diseases can still occur when environmental conditions remain conducive for disease development (McDonald et al., 2006; Delvalle et al., 2011). Fungicide applications are often required when environmental conditions both hinder creeping bentgrass growth and promote disease development.

Disease management using fungicides on creeping bentgrass greens consists of rotating between differing modes of action to help reduce fungicide resistance and to control a broader spectrum of diseases (Latin, 2011). This is referred to as fungicide programming. Fungicide programs are developed to provide a scheduled application approach to combat diseases when they are most commonly observed in the field and are only be applicable to areas that share a similar climatic zone. Fungicide programs have been developed for several agricultural crops including potato (*Solanum tuberosum* L.), wheat (*Triticum aestivum* L.), peanut (*Arachis hypogaea* L.), and grapes (*Vitis vinifera* L.) in addition to turfgrass (Rideout et al., 2012; Kirk et al., 2011; Wilcox and Riegel, 2010; Hagan et al., 2009; Vincelli et al., 2009). Research conducted in transition zone states such as Oklahoma, Kentucky, and Tennessee has reported greater disease control and visual quality of creeping bentgrass and annual bluegrass putting greens when multiple strobilurin fungicides applications are incorporated within a summer fungicide program (Smith and Walker, 2009; Vincelli et al., 2009).

Azoxystrobin was the first strobilurin fungicide introduced to the turfgrass marketplace in 1996 (Köhle, 2002). As of the 2012, trifloxystrobin, pyraclostrobin, and fluoxastrobin are all labeled for use in turf as well (Latin, 2011). These fungicides are often applied to creeping bentgrass putting greens in warm, humid environments to control diseases such as brown patch and anthracnose (Latin, 2011). Strobilurin fungicides inhibit mitochondrial respiration in fungi by binding at the ubiquinone site located within the cytochrome bc_1 complex of the mitochondrial membrane (Bartlett et al., 2001). These fungicides are unique in that they are derived from a natural wood-rotting fungus (*Strobilurus tenacellus*) and are classified as reduced-risk fungicides by the Environmental Protection Agency (EPA) (Köhle et al., 2002).

Strobilurin fungicides are labeled to control several diseases affecting creeping bentgrass putting greens during summer conditions, including brown patch. Strobilurin fungicide efficacy for brown patch control is well documented (Uddin et al., 2010; Soika and Tredway, 2010). Efficacy against dollar spot, anthracnose, and *Pythium* root diseases can vary among strobilurins (Kerns et al. 2009). In addition, pyraclostrobin is labeled for disease control as well as promotion of plant health benefits. (Anonymous, 2010).

Strobilurin fungicides have been shown to affect an array of physiological processes in several cropping systems. Wu and Tiedemann (2002) reported a 50 to 60% reduction from fumigated ozone injury in spring barley (*Hordeum vulgare* L.) plants in a greenhouse after a foliar spray of azoxystrobin. Wheat (*Triticum aestivum* L.) plants treated with the strobilurin kresoxim-methyl showed a 50% reduction in ethylene formation compared to non-treated plants (Grossmann and Retzlaff, 1997). A partial inhibition of ethylene formation may explain the significant yield improvements observed in several cropping systems in the field after applications of strobilurin fungicides (Ammermann et al., 2000; Godwin et al., 2000; Bryson, 2000). However, other studies suggest this increase in grain yield after strobilurin fungicide applications is much less pronounced in low disease pressure environments (Swoboda and Pedersen, 2009; Weisz et al., 2011). In turfgrass, Brosnan et al. (2010) observed significant increases in total root length and root biomass in 'Penn A-1' creeping bentgrass plants treated with pyraclostrobin compared to azoxystrobin or non-treated creeping bentgrass plants. However this effect was not observed on 'Penncross' creeping bentgrass. In this study, the creeping bentgrass plants were grown in a greenhouse and were subjected to temperature and moisture stress. However, at no time was turfgrass quality statistically different among treatments.

Strobilurin fungicides exhibit efficacy for disease control. In research trials, disease control efficacy is often the primary means by which strobilurins are evaluated while effects on turfgrass quality are secondary. Little published data exists where a direct comparison is made between the performance of azoxystrobin, pyraclostrobin, fluoxastrobin, and trifloxystrobin when integrated in a summer fungicide program. Furthermore, data describing the plant physiological effects of these fungicides on turfgrass have never been closely examined in the field. The objective of this study was to examine disease control efficacy and plant physiological effects of summer fungicide programs featuring azoxystrobin, pyraclostrobin, trifloxystrobin, and fluoxastrobin on a creeping bentgrass putting green in the transition zone.

MATERIALS AND METHODS

Research site. This study was conducted in 2011 and 2012 at Ruggles Ferry Golf Club (Strawberry Plains, TN) on a 'Dominant Southern' (a blend consisting of SR1120 and SR 1119) creeping bentgrass putting green. The putting green was established according to United States Golf Association specifications (United States Golf Association, 2007). This field trial was conducted in full sunlight and mowed 4 times weekly at 3 mm with a reel mower. Irrigation was applied during morning hours as needed to prevent wilt. The putting green received 25 kg N ha^{-1} of urea per month during the spring, fall, and winter months. During summer of both years 10 kg N ha^{-1} of urea was applied biweekly. The trial area was aerated and topdressed with sand each spring and fall each year. Chlorantraniliprole (Acelepryn; Dupont Crop Protection, Wilmington, DE) was applied preventively during spring of both years to reduce insect populations. Triticonazole (Trinity; BASF Corporation, Research Triangle Park, NC) was applied during early May of both years to reduce disease severity before trial initiation. No additional environmental stress factor was artificially imposed and no inoculation procedure was administered.

Treatments. Treatments were arranged in a randomized complete block design with four replications. Plots measured 0.9 x 1.2 m. Fungicide programs were initiated on 31 May in 2011 and 1 June in 2012 (Table 2.1). Strobilurin treatments included 1) azoxystrobin (Heritage TL; Syngenta Crop Protection, Greensboro, NC) at 0.61 kg ai ha^{-1}; 2) pyraclostrobin (Insignia SC; BASF Corporation, Research Triangle Park, NC) at 0.55 kg ai ha^{-1}; 3) fluoxastrobin (Disarm; Arysta LifeScience North America LLC, Cary, NC) at 0.55 kg ai ha^{-1}; 4) trifloxystrobin (Compass; Bayer Environmental Science, Research Triangle Park, NC) at 0.38 kg ai ha^{-1}; and 5) non-treated control. The rates of application represent the high labeled rates for each of the strobilurin fungicides. These treatments made up the 2nd and 5th application within each fungicide program. All treatments within each fungicide program were the same except for the strobilurin treatments. Treatments were applied as a foliar spray using a spray boom equipped with two, flat-fan, nozzles (8004EVS; Spraying Systems Co., Roswell, GA) calibrated to deliver a spray volume of 807 L per ha^{-1}.

Data collection. Turfgrass quality was assessed immediately prior to each spray application. Turfgrass quality was evaluated by estimating the overall color, density, uniformity, growth, and leaf texture using a 1 to 9 scale where 1 = brown or dead turf and 9 = exceptional quality. A value of 6 was considered to be the minimum acceptable level of turfgrass quality for industry standards. Turfgrass quality data were collected by the same researcher in both experimental runs.

Spectral reflectance of the canopy was measured immediately prior to each spray application using a Crop Circle™ reflectance spectrometer (ACS-470; Holland Scientific, Lincoln, NE). This model had a built-in light sensor that simultaneously emits visible and near infrared light onto the turf canopy. These sensors were fitted onto a golf push cart and suspended approximately 0.75 m above the canopy surface. This apparatus was pushed across the center of the plot to obtain normalized difference vegetation index (NDVI) and ratio vegetation index (RVI) values for each treatment. NDVI and RVI were logged with a GeoSCOUT DIS Datalogger (GLS-400; Holland Scientific, Lincoln, NE).

Analysis of percent turfgrass cover was quantified using digital image analysis (DIA). Digital images were captured using a Canon G12 (Canon G12; Canon Inc., Japan) digital camera. In 2011, the camera was mounted inside a 0.28 m^2 box equipped with four fluorescent light bulbs. In 2012, the 0.28 m^2 light box was equipped with four light-emitting diodes (LED). Digital images were captured immediately prior to each spray application. Image size in this experiment was 307,200 pixels. Digital images were analyzed using SigmaScan Pro (Version 5.0; SPSS Inc., Chicago, IL) software similar to the methods of Richardson et al. (2001). Pixels classified as green turf expressed a hue range of 45^o to 120^o, with saturation defined between 0% and 100%.

Brown patch and dollar spot severity were visually assessed when symptoms were present in the trial area. Brown patch was evaluated as percent turf area infected. Dollar spot was evaluated by counting the number of dollar spot foci per plot. No other confirmed diseases were observed during the trial duration in either year.

A root morphology assessment was conducted at the termination of the trial period after both experimental runs. A soil profiler (Soil profiler; Forestry Suppliers Inc., Jackson, MS) was used to extract 14 soil profiles from each plot. Soil profiles measured 25cm^2 and were pulled from a depth of 18 cm into the rootzone. Soil profiles were gently rinsed with water to remove sand and organic contaminants. In 2011, the remaining roots were scanned and analyzed for total root length, root surface area, root diameter and root volume using the WinRhizo Pro system (WinRhizo Pro; Regent Instruments Inc., Canada). Procedures were similar to those outlined in Beasley and Branham (2007) and Brosnan et al. (2010). WinRhizo measurements were not conducted in 2012. To determine root biomass, roots were placed inside a drying oven (LR-271C, Grieve Corporation, Round Lake, IL) at 105 ^{o}C for 48 h and weighed. Roots were transferred to a muffle furnace (F62735, Thermo Scientific/Thermolyne, Dubuque, IA) at 450 ^{o}C for 4 h. Biomass was calculated by subtracting the remaining ash weight from the initial dry weight.

Statistical analyses. Data were subjected to analysis of variance (ANOVA) using PROC MIXED with code generated by the DANDA macro (Saxton, 2010) in SAS (Statistical Analysis Software, Inc., Cary, NC). Means were separated using Fisher's protected least significant difference (LSD) at α=0.05. A combined ANOVA was conducted to test for significant interactions between experimental runs. Significant experimental run x treatment interactions were detected for all measured parameters so data were analyzed and presented separately. The area under the progress curve (AU(x)PC) was calculated for each of the evaluation parameters where (x) is a parameter of interest. The (AU(x)PC) describes the course of increase in (x) parameter over time (Campbell and Madden, 1991). The area under the progress curve was selected to supplement individual assessment dates as a quantifiable measure how the fungicide programs performed as a whole across the entire season.

RESULTS

Turfgrass Quality

In 2011, significant differences in turfgrass quality were only detected on a single rating date (Table 2.2). On this date, fungicide programs featuring trifloxystrobin exhibited significantly lower turfgrass quality than all other fungicide programs and the non-treated control. However, at no time was turfgrass quality considered unacceptable (<6.0) throughout the duration of the trial period (data not presented). No significant differences were detected in the area under the turfgrass quality progress curve (Table 2.3).

In 2012, the non-treated control had significantly lower turfgrass quality compared to all fungicide programs from 42 DAIT until the end of the trial (Table 2.4). Significant differences among fungicide programs were only detected on the last rating date (70 DAIT). On this date, fungicide programs featuring trifloxystrobin had significantly lower turfgrass quality than pyraclostrobin (SC) (Table 2.5). At no time was turfgrass quality considered unacceptable (< 6.0) on any rating date. Significant differences in the area of the turfgrass quality progress curve were observed in 2012. The non-treated control resulted in significantly lower turfgrass quality compared to all other treatments. No differences among fungicide programs were observed using this analysis (Table 2.3).

Turfgrass Cover

Similar to visual quality, no significant differences were observed for turfgrass cover between treatments on any rating date, nor were differences detected in the area under the turfgrass cover progress curve in 2011. In 2012, significant differences were observed in turfgrass cover from 42 to 70 DAIT (Table 2.4). From 42 to 70 DAIT, treatments including azoxystrobin, pyraclostrobin, and fluoxastrobin had significantly greater turfgrass cover than the non-treated control. Trifloxystrobin had significantly greater percent turfgrass cover compared to the non-treated control on 42 and 70 DAIT. On 56 DAIT, trifloxystrobin and the non-treated control were not statistically different (data not presented). Pyraclostrobin and azoxystrobin had significantly greater turfgrass cover compared to all treatments when analyzing the area under the turfgrass cover progress curve. Fluoxastrobin was not significantly different from trifloxystrobin, but was significantly greater than the non-treated control (Table 2.3).

Spectral Reflectance

In 2011, no significant differences in NDVI, RVI, or the area under the respective progress curves were observed on any rating date. In 2012, significant differences on 56 and 70 DAIT occurred for both NDVI and RVI. The non-treated control had significantly lower NDVI and RVI than all fungicide programs on these assessment dates. Significant differences among fungicide programs only occurred at 56 DAIT. On this rating date, azoxystrobin programs had significantly greater NDVI than programs that included trifloxystrobin. Furthermore, azoxystrobin and pyraclostrobin were statistically similar and had significantly greater RVI than programs that included trifloxystrobin (data not presented). However, no significant differences were observed for the area under the NDVI or RVI progress curves (Table 2.3).

Root analysis

In both experimental runs, strobilurin fungicide programs did not significantly impact any rooting parameter measured compared to the non-treated control (Table 2.6). Brosnan et al. (2010) reported greater visual root length on ‘Penn A-4’ creeping bentgrass in a greenhouse experiment when treated with pyraclostrobin, though these differences were relatively small (<10%). In comparison, this field research was conducted on established creeping bentgrass plants grown on a mature rootzone. The range in total root length in this experiment between samples was from 186 cm to 4,224 cm (data not presented). The large variability could be the result of a number of factors. Differences in soil moisture retention or other isolated edaphic stressors may have led to increased variability in root length. Another potential cause is fungi including sub-clinical root infecting diseases such as *Pythium* root diseases. Other diseases such as fairy ring can render the soil impermeable to water that may adversely affect root growth (Dernoeden, 2002).

Disease severity

Disease pressure was exceptionally low throughout both experimental runs. In 2011, trace amounts of brown patch (1.3 %) were observed in the non-treated control on a single rating date (70 DAIT); however no significant differences occurred between treatments. In 2012, significant differences in brown patch severity were observed 42 and 56 DAIT (Table 2.7). On these dates brown patch severity in the non-treated control reached 6 to 8%, significantly greater than all other treatments. All fungicide programs did not exhibit observable symptoms associated with brown patch and dollar spot in either year. In 2012, these fungicide programs also had significantly lower brown patch compared to the non-treated control as measured by the area under the brown patch progress curve (Table 2.7).

CONCLUSION

In 2011, visual quality, turfgrass cover, and spectral reflectance measurements gradually declined within the trial area on each successive date. However, strobilurin fungicide programs did not statistically improve any of these measured parameters compared to the non-treated control. The amount of disease within the trial area was extremely low throughout the trial duration and may be a contributing factor for the lack of observable differences in visual quality, percent cover, and spectral reflectance measurements.

In 2012, significant differences in visual quality and percent cover were detected 42 DAIT and continued through the remainder of the trial period. Spectral reflectance measurements were significantly different beginning 56 DAIT. Diseases such as brown patch were more prevalent during 2012 trial season. Significant differences in disease severity were observed 42 and 56 DAIT.

Prior investigations have supported these research findings. Weiz et al. (2011) and Swoboda and Pedersen (2009) reported minimal significant differences in plant physiological effects or yield in a low disease pressure environment using strobilurin fungicides. In comparison, this field research reported no differences in any parameter prior to a disease occurrence within the trial area. Brosnan et al. (2010) observed significant increases in root length and density in ‘Penn A-1’ creeping bentgrass after applications of pyraclostrobin compared to the non-treated control in a greenhouse environment. However, this field study featured established creeping bentgrass plants grown on a mature sand rootzone in the field. Established rootzones are prone to a number of isolated edaphic stressors that may cause increased variability in root structure. Wilkinson and Miller (1978) reported significant changes in the particle size distribution of a rootzone over two year period. These changes included a decrease of medium sized (0.25 – 0.05 mm) sand particles and an increase in very course (1 – 2 mm) and clay (< 0.002 mm) sand particles. The authors proposed the increase in very coarse particle size was due to smaller particles binding together to create coarser particles. Using a scanning electron microscope the authors observed an organic waxy coating binding these particles together. The waxy coating was determined to be caused by fairy ring fungal colonization. These areas of a putting green often result in localized dry spots. Localized dry spot or other hydrophobic sands can result in significantly less total root length at the 0 – 8 cm root depth (Karnok and Tucker, 2001). This may have been a contributing factor to the variance in root morphology assessments during this experiment.

The physiological responses that have been observed after strobilurin applications such as inhibition of ethylene, increased anti-oxidant activity, and hormonal shifts may still have been taking place during this experiment (Grossmann and Retzlaff, 1997; Köhle et al., 2002; Jabs et al., 2002). However, the practical implication of these processes remains unclear under field conditions. In this experiment we did not have consistent field observable differences in physiological effects. This may be a result of implementing cultural techniques that promote a healthier growing environment for creeping bentgrass putting greens. Routine mowing, sand topdressing, aerification, and proper nutrient and water management practices may be a contributing factor for the lack of significant differences in plant physiological effects when disease pressure is low.

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APPENDIX

TABLES AND FIGURES

Table 2.1. Summer fungicide program integrated with strobilurin fungicides.

Application interval†	Fungicide‡	Fungicide class	Rate (kg ha^{-1})
1	Iprodione	Dicarboximide	3.05
2,5	Strobilurin§	Quinone outside inhibitor	
3	Thiophanate-methyl	Benzimidazole	3.16
	Chlorothalonil	Benzonitrile	12.65
4	Fosetyl-aluminum	Phosphonate	9.76
	Chlorothalonil	Benzonitrile	8.05

† Applications were initiated in Strawberry Plains, TN, on 1-JUN in 2011 and 31-May in 2012 on a'Dominant Southern' creeping bentgrass (*Agrostis stolonifera* L.) putting green. Subsequent applications were made on 14-day intervals.
‡ Fungicides were applied using two, flat-fan, nozzles calibrated to deliver a spray volume of 807 L per ha^{-1}.
§ Azoxystrobin, pyraclostrobin, fluoxastrobin, and trifloxystrobin were applied at rates 0.55, 0.61, 0.55, and 0.38 kg ha^{-1}, respectively, on the 2^{nd} and 5^{th} application interval.

Table 2.2 Analysis of variance for turfgrass quality, turfgrass cover, normalized difference vegetation index (NDVI), and ratio vegetation index (RVI) on a 'Dominant Southern' creeping bentgrass (*Agrostis stolonifera* L.) putting green subjected to various strobilurin fungicide programs in 2011 in Strawberry Plains, TN.

			Days after initial treatment				
		df	**14**	**28**	**42**	**56**	**70**
Turfgrass Quality	Treatment	4	NS†	NS	NS	*	NS
Turfgrass Cover	Treatment	4	*	NS	NS	NS	NS
NDVI	Treatment	4	NS	NS	NS	NS	NS
RVI	Treatment	4	NS	NS	NS	NS	NS

* Significant at the $p \leq 0.05$ level.

† NS, not significant.

Table 2.3. Analysis of area under the (X) progress curve for visual turfgrass quality, normalized difference vegetation index (NDVI), and ratio vegetation index (RVI), and percent turfgrass cover following applications of various strobilurin fungicide programs in 2011 and 2012 in Strawberry Plains, TN, on a 'Dominant Southern' creeping bentgrass (*Agrostis stolonifera* L.) putting green.

Experimental year	Fungicide Program	Area Under the (X) Progress Curve			
		Turfgrass quality	NDVI	RVI	Turfgrass cover
2011	Trifloxystrobin	388.3	38.2	304.9	5579.4
	Fluoxastrobin	391.3	38.9	317.1	5584.6
	Pyraclostrobin	390.6	38.4	306.8	5584.8
	Azoxystrobin	390.9	38.8	315.6	5583.3
	Untreated check	389.4	38.6	312.6	5583.9
	$LSD_{0.05}$	NS†	NS	NS	NS
2012	Trifloxystrobin	388.9	38.8	315.2	5145.0
	Fluoxastrobin	390.3	38.8	313.1	5325.2
	Pyraclostrobin	390.3	39.2	322.1	5541.0
	Azoxystrobin	390.8	39.0	317.4	5580.1
	Untreated check	378.1	38.2	308.7	4983.7
	$LSD_{0.05}$	2.6	NS	NS	207.6

† NS, not significant.

Table 2.4. Analysis of variance for turfgrass quality, turfgrass cover, normalized difference vegetation index (NDVI), and ratio vegetation index (RVI) following applications of various strobilurin fungicide programs in 2012 in Strawberry Plains, TN, on a 'Dominant Southern' creeping bentgrass (*Agrostis stolonifera* L.) putting green subjected to various strobilurin fungicide programs in 2012.

			Days after initial treatment				
		df	**14**	**28**	**42**	**56**	**70**
Turfgrass Quality	Treatment	4	NS†	NS	**	***	***
Turfgrass Cover	Treatment	4	NS	NS	*	***	***
NDVI	Treatment	4	NS	NS	NS	***	**
RVI	Treatment	4	NS	NS	NS	***	***

* Significant at the $p \leq 0.05$ level.
** Significant at the $p \leq 0.01$ level.
*** Significant at the $p \leq 0.001$ level.
† NS, not significant.

Table 2.5. Visual measurements of turfgrass quality under various strobilurin fungicide programs in 2012. Fungicide programs were located in Strawberry Plains, TN, on a 'Dominant Southern' creeping bentgrass (*Agrostis stolonifera* L.) putting green.

Fungicide Program	Turfgrass quality†				
	Days after initial treatment				
	14	28	42	56	70
Trifloxystrobin	7‡	7	7	6.9	6.8
Fluoxastrobin	7	7	7	6.9	6.9
Pyraclostrobin	7	7	6.9	6.9	6.9
Azoxystrobin	7	7	7	6.9	6.9
Untreated check	7	7	6.7	6.5	6.6
$LSD_{0.05}$	NS§	NS	0.15	0.09	0.07

† Turfgrass quality was quantified by using a 1 to 9 scale where 1 = brown or dead turf and 9 = exceptional quality. A value of 6 is considered to the minimum acceptable level of quality for industry standards.
‡ Values are means of four replications.
§ NS, not significant.

Table 2.6. Total root length, root surface area, root length density, root volume, and root biomass of a 'Dominant Southern' creeping bentgrass (*Agrostis stolonifera* L.) putting green treated with various strobilurin fungicide programs in 2011 in Strawberry Plains, TN.

Fungicide program	Total Root length	Root surface area	Root diameter	Root volume	Root biomass
	cm†	cm^2	mm	cm^3	g
Trifloxystrobin	1760‡	134	0.232	0.83	0.039
Fluoxastrobin	1682	125	0.229	0.75	0.037
Pyraclostrobin	1768	136	0.233	0.85	0.039
Azoxystrobin	1965	154	0.238	0.98	0.042
Untreated check	2045	172	0.258	1.18	0.045
$LSD_{0.05}$	NS§	NS	NS	NS	NS

† 14 soil profiles were removed from each plot. Profiles were measured separately and then averaged across the entire plot.
‡ Values are means of four replications.
§ NS, not significant.

Table 2.7. Analysis of variance for percent brown patch for individual dates and the area under the brown patch progress curve (AUBPPC) on 'Dominant Southern' creeping bentgrass (*Agrostis stolonifera* L.) putting green subjected to various strobilurin fungicide programs in 2011 and 2012 in Strawberry Plains, TN.

			Days after initial treatment					
		df	**14**	**28**	**42**	**56**	**70**	**AUBPPC**
2011	Treatment	4	NS†	NS	NS	NS	NS	NS
2012	Treatment	4	NS	NS	***	***	NS	***

*** Significant at the $p \leq 0.001$ level.
† NS, not significant.

CHAPTER III

PHYSIOLOGICAL EFFECTS OF CREEPING BENTGRASS PUTTING GREENS IN RESPONSE TO REPEATED STROBILURIN FUNGICIDE APPLICATIONS IN THE FIELD

The intent of this manuscript is to publish articles in the peer-reviewed literature. This work is based on contributions by Jesse Benelli, Brandon Horvath, Jim Brosnan, and Dean Kopsell:

My primary contributions to this paper include (i) designing and conducting the experiments, (ii) analyzing and interpreting data, (iii) literature review, and (iv) writing the manuscript.

ABSTRACT

Applications of strobilurin fungicides have increased stress tolerance and yield of corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) plants grown in the field and improved rooting of creeping bentgrass (*Agrostis stolonifera* L.) in controlled greenhouse conditions. A two-year experiment was conducted to determine if applications of pyraclostrobin (0.55 kg ha^{-1}), azoxystrobin (0.61 kg ha^{-1}), fluoxastrobin (0.55 kg ha^{-1}), and trifloxystrobin (0.38 kg ha^{-1}) could mitigate environmental stress on a 'Dominant Southern' creeping bentgrass putting green. Experimental units were arranged in a split-plot randomized complete block design with XX replications in 2011 and 2012. Whole plots included a fungicide mixture containing chlorothalonil and iprodione applied every 14 days or no fungicide treatment. Sub-plots were treated with strobilurin fungicides (azoxystrobin, pyraclostrobin, fluoxastrobin, and trifloxystrobin) and a non-treated control. Turfgrass quality, normalized difference vegetation index (NDVI), ratio vegetation index (RVI), and percent turfgrass cover data were collected 14, 28, 42, 56, 70, and 84 days after initial treatment. Soil profiles were removed from each subplot at

the termination of the trial period to assess visual root length. Significant differences were not detected for any measured parameter on any assessment date. While applications of strobilurin fungicides affect creeping bentgrass physiology during summer stress, field observation of these effects may be dependent on a number of factors that are currently unknown.

INTRODUCTION

Creeping bentgrass (*Agrostis stolonifera* L.) is the predominant grass species used on golf course putting greens throughout much of the United States (Lyman et al., 2007). In the transition zone, creeping bentgrass can maintain its green color throughout winter and provides exceptional acceptable quality during spring and fall months (Xu and Huang, 2003). The quality and health of creeping bentgrass is most compromised when prolonged heat occurs during summer months (Dernoeden, 2002).

Creeping bentgrass is prone to a number of summer stress maladies including heat stress, ultraviolet light radiation, and pathogen attack (Dernoeden, 2002). These stressors can impair many of the physiological processes of higher plants including photosynthesis, respiration, water regulation, and hormonal balance (Wahid et al., 2007). These cellular impairments can result reduce the playability and quality of creeping bentgrass putting greens. Proper management practices to mitigate injury and to lessen disease severity are important for sustaining this cool season plant. One of those management practices is fungicide selection.

Strobilurin fungicides are broad-spectrum disease control products that were first registered for turfgrass use in 1996 with the introduction of azoxystrobin (Latin, 2011). By 2012, the list of available strobilurin fungicides has broadened to include trifloxystrobin, pyraclostrobin, and fluoaxastrobin (Latin, 2011). These fungicides are often applied to creeping bentgrass in warm, humid environments to control diseases such as brown patch (*Rhizoctonia solani* J.G. Kühn) and anthracnose (*Colletotrichum cereale* sensu lato Crouch, Clarke and Hillman) (Wong et al., 2006).

Strobilurin fungicides inhibit mitochondrial respiration in fungi by binding to the ubiquinone site located within the cytochrome c oxidoreductase complex (bc_1) of the mitochondrial membrane (Bartlett et al., 2001). This results in the inactivation of the ubiquinol oxidase enzyme causing a blockage of electron transport that inhibits the production of ATP, the main source of energy needed for fungal growth (Bartlett et al., 2001). Recently, pyraclostrobin has been labeled by the Environmental Protection Agency (EPA) for plant health benefits in addition to disease control (Anonymous, Insignia SC fungicide product label, 2010).

Strobilurin fungicide effects on crop physiology are well documented. Wu and Tiedemann (2001) demonstrated that strobilurin fungicides significantly reduced physiological leaf spot symptoms on spring and winter barley (*Hordeum vulgare* L.) when exposed to elevated levels of ozone. In this study, strobilurin fungicides increased anti-oxidant production leading to a reduction in oxidative stress from free radicals in the strobilurin treated barley plants that favors rapid quenching of these free radicals. Strobilurin fungicides may also indirectly enhance nitrogen assimilation in higher plants (Köhle et al., 2002). Glaab and Kaiser (1999) found that a buffer solution of the strobilurin kresoxim-methyl prevents the inactivation of nitrate reductase (NR) in spinach leaf discs while leaf discs without kresoxim-methyl had a 20% decrease in NR.

Applications of strobilurin fungicides have been suggested to cause a "greening" effect in agricultural crops. This greening effect may be due in part to ethylene inhibition after application. Grossmann and Retzlaff (1997) demonstrated that wheat (*Triticum aestivum* L.) plants treated with kresoxim-methyl had a 50% reduction in ethylene formation compared non-treated wheat plants. A delay in leaf senescence has also been suggested to lengthen the grain filling period of corn (*Zea mays* L.) and wheat (Ammermann et al., 2000; Godwin et al., 2000; Bryson, 2000). It is hypothesized that this increase in grain yield is due to the prolonged photosynthetic ability in the lower (older) leaves (Grossman et al., 1999).

Strobilurin fungicide applications on creeping bentgrass could facilitate hormonal shifts that may aid in abiotic stress tolerance. An accumulation of ethylene in creeping bentgrass leaves has been associated with reduced leaf senescence, chlorophyll content, and greater turf quality of creeping bentgrass during high temperature stress (Xu and Huang, 2009). Applications of strobilurin fungicides may also have an effect on creeping bentgrass similar to low doses of the plant hormone auxin. Brosnon et al. (2010) observed that applications of the strobilurin pyraclostrobin increased rooting in 'Penn A-1' creeping bentgrass. However, this effect was not observed with 'Penncross' creeping bentgrass. Significant differences in turfgrass quality were not observed during the study. Auxins have been associated with adventitious root growth in higher plants (Taiz and Zeiger, 1998)

Strobilurin fungicides are commonly used for disease control on creeping bentgrass greens during summer; however the physiological effects associated with these fungicides have not been carefully examined in the field. The objective of this project was to evaluate physiological effects of strobilurin fungicide applications on creeping bentgrass under field conditions during summer stress.

MATERIALS AND METHODS

Research site. A 2-year field experiment was conducted in 2011 and 2012 at the East Tennessee Research and Education Center (Knoxville, TN; 35° 57' N Lat.) on a 'Dominant Southern' creeping bentgrass putting green. The putting green was established according to United States Golf Association specifications (United States Golf Association, 2007) with a rootzone mix of 85% sand and 15% sphagnum peat moss by volume. Field trials were conducted in full sunlight and mowed 4 times weekly at 3 mm. Irrigation was applied as needed to prevent wilt. In 2011 and 2012, the research area received 25 kg N ha^{-1} of urea per month during the spring, fall, and winter months. During summer of both years 10 kg N ha^{-1} of urea was applied biweekly. The trial areas were aerated and top dressed with sand each spring and fall in 2011 and 2012. Chlorothalonil (Daconil Ultrex; Syngenta Crop Protection, Greensboro, NC) was applied on May 1st during both years to minimize pathogen activity within the trial area. Chlorantraniliprole (Acelepryn; Dupont Crop Protection, Wilmington, DE) was applied preventively during spring of both years to reduce black cutworm (*Agrotis ipsilon* Hufnagel) populations.

Treatments. Treatments were arranged in a randomized complete block design in a split-plot arrangement with four replications. Whole plots were overspray treatments. Overspray treatments included applications of a fungicide mixture containing chlorothalonil (Daconil Ultrex; Syngenta Crop Protection, Greensboro, NC) and iprodione (26GT; Bayer Environmental Science, Research Triangle Park, NC) at 8.05 and 3.05 kg ai ha^{-1}, respectively, or no fungicide treatment. Subplot treatments were fungicides and included 1) azoxystrobin (Heritage TL; Syngenta Crop Protection, Greensboro, NC) at 0.61 kg ai ha^{-1}; 2) azoxystrobin (Heritage WDG; Syngenta Crop Protection, Greensboro, NC) at 0.61 kg ai ha^{-1}; 3) pyraclostrobin (Insignia SC; BASF Corporation, Research Triangle Park, NC) at 0.55 kg ai ha^{-1}; 4) pyraclostrobin (Insignia WG; BASF Corporation, Research Triangle Park, NC) at 0.55 kg ai ha^{-1}; 5) fluoxastrobin (Disarm; Arysta LifeScience North America LLC, Cary, NC) at 0.55 kg ai ha^{-1}; 6) trifloxystrobin (Compass: Bayer Environmental Science, Research Triangle Park, NC) at 0.38 kg ai ha^{-1}; and 7) non-treated control. All treatments were applied using a CO_2-pressurized sprayer calibrated to deliver 815 L ha^{-1}. The spray boom was equipped with two, flat-fan, nozzles (8004EVS; Spraying Systems Co., Roswell, GA) spaced 45 cm apart. Treatments were initiated on 31 May 2011 and 1 June 2012. Treatments were applied to the same experimental area in both years.

Data collection. Turfgrass quality was visually evaluated prior to each spray application by assessing the overall color, density, uniformity, growth, and leaf texture of each treated plot on a 1 to 9 scale, where 1 = brown or dead turf and 9 = exceptional quality. A value of 6 is considered to the minimum acceptable level of quality for industry standards. The same researcher evaluated turfgrass quality in both experimental runs.

Spectral reflectance of the turfgrass canopy was measured immediately prior to each spray application with a Crop Circle™ reflectance spectrometer (ACS-470; Holland Scientific, Lincoln, NE). This model has a built-in light sensor that simultaneously emits visible and near infrared light onto the turfgrass canopy. The sensor was fitted onto a golf push cart and suspended 0.75 m above the turfgrass canopy. This apparatus was pushed across the center of the plot to obtain normalized difference vegetation index (NDVI) and ratio vegetation index (RVI) values for each treatment. NDVI and RVI were logged with a GeoSCOUT GIS Datalogger (GLS-400; Holland Scientific, Lincoln, NE).

Turfgrass cover was quantified using digital image analysis. Digital images were captured using a Canon G12 (Canon G12; Canon Inc., Japan) digital camera. In 2011, the camera was mounted inside a 0.28 m^2 box equipped with four fluorescent light bulbs. In 2012, the 0.28 m^2 light box was equipped with four light-emmitting diodes (LED). Digital images were captured immediately prior to each spray application. Image size in this experiment was 307,200 pixels. Digital images were analyzed similar, with the exception of image size, to the methods of Richardson et al. (2001) using SigmaScan Pro software (Version 5.0; SPSS Inc., Chicago, IL). Pixels classified as green turf had a hue range of range of 45^o to 120^o with saturation defined between 0% and 100%.

Disease severity within the trial area was assessed when symptoms were present prior to each spray application. Brown patch (*Rhizoctonia solani* Kühn) and cyanobacteria (*Oscillatoria spp.*) severity was quantified by measuring the percentage of each plot infected. Dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) was evaluated by counting the number of dollar spot infection centers per plot.

Root length was assessed at the termination of the trial period. A soil profiler (Soil profiler, Forestry Suppliers Inc., Jackson, MS) was used to extract three soil profiles from each sub-plot. Soil profiles measured 25 cm^2 and were pulled from a depth of 18 cm into the rootzone. The longest root extending from the soil profile base of the crown was measured.

Statistical analysis. Data were subjected to analysis of variance (ANOVA) using PROC MIXED with code generated by the DANDA macro (Saxton, 2010) in SAS

(Statistical Analysis Software, Inc., Cary, NC). Means were separated using Fisher's protected least significant difference (LSD) at $\alpha = 0.05$. A combined ANOVA was conducted to test for significant interactions between experimental runs. The area under the progress curve (AU(x)PC) was calculated for each of the evaluation parameters where (x) is a parameter of interest. The (AU(x)PC) describes the course of increase in (x) parameter over time (Campbell and Madden, 1991). The area under the progress curve was used to supplement individual assessment dates as a quantifiable measure how repeated applications of strobilurin fungicides on creeping bentgrass performed as a whole across the entire trial period. Significant treatment-by-year interactions were not detected in turfgrass quality, turfgrass cover, NDVI, RVI, visual root length, dollar spot, brown patch, or cyanobacteria data. Thus, data for each parameter were combined for clarity.

RESULTS

Turfgrass Quality

Turfgrass quality progressively declined throughout the trial period (Table 3.1). However, no significant overspray-by-fungicide interactions were detected on any assessment date (Table 3.2). Fungicide was only significant on one rating date (42 DAIT) with trifloxystrobin having significantly lower turfgrass quality compared to all other treatments (data not shown). Turfgrass quality did not fall below an acceptable level (< 6) at any time (Table 3.1). No significant differences were detected for the area under the turfgrass quality progress curve (Table 3.3).

Significant differences in turfgrass quality between whole plots were detected on 14, 42, 56, and 84 DAIT (Table 3.2). Differences in quality may be explained by the control of additional biotic agents beyond those controlled by the strobilurin fungicides during the trial period. Cyanobacteria severity was lower on whole plots receiving iprodione + chlorothalonil on all rating dates. Similarly, these whole plots contained fewer dollar spot foci from 42 to 84 DAIT compared to the non-treated whole plot (Table 3.4).

Findings of this field experiment supported those reported by Brosnan et al. (2010) following strobilurin applications to creeping bentgrass in mini-rhizotron culture in a greenhouse. At no time in that experiment did strobilurin fungicide application improve turfgrass quality compared to a non-treated control.

Turfgrass Cover

No significant overspray-by-fungicide difference were detected at any time (Table 3.1). Similarly, no significant overspray-by-fungicide differences were detected in area under the percent cover progress curve data (Table 3.3). However, significant differences in turfgrass cover were detected between whole plots 28 DAIT (Table 3.2). On this date, overspray whole plots exhibited three percent more turfgrass cover compared to the non-treated whole plot. Significant differences between whole plots were not detected on any other rating date.

Spectral Reflectance

NDVI progressively declined for all treatments during the summer months (Table 3.5) with RVI data following a similar trend (data not shown). No significant overspray-by-fungicide interactions were detected in NDVI or RVI data on any assessment date. Similarly, no significant overspray-by-fungicide interactions were detected for the area under the NDVI or RVI progress curves (Table 3.3).

Significant differences in spectral reflectance between whole plots were limited. Oversprayed whole plots had significantly greater NDVI (on two rating dates) and RVI (one rating date) compared to the untreated whole-plot (Table 3.6). Limited whole-plot differences may be due in part to the presence of cyanobacteria within the non-treated whole plot. NDVI and RVI detect chlorophyll reflectance produced by higher plants including turfgrass (Bell et al., 2004). Cyanobacteria also have chloroplasts producing chlorophyll that could be detected by reflectance instruments such as NDVI (Hu, 2009).

Visual Root Length

No significant differences in visual root length were observed this study. Visual root length ranged from 9.5 to 10.2 cm within all subplots (Table 3.7). These reports differ from those of Brosnan et al. (2010). They reported greater visual root length on ‘Penn A-4’ creeping bentgrass in a greenhouse experiment following treatment with pyraclostrobin at 0.55 kg ai ha^{-1}; however, these increases were relatively small (<10%) compared to non-treated plants. Furthermore, the researchers observed no significant differences in visual root length were observed for ‘Penncross’ creeping bentgrass indicating that effects of pyraclostrobin on creeping bentgrass root growth may vary by cultivar.

Dollar spot

Dollar spot pressure was low (< 10.0 infection centers in all subplot treatments) throughout the trial period. A significant overspray-by-fungicide was observed 84 DAIT (Table 3.4). On this rating date, plots receiving no fungicide treatment contained 9.75 dollar spot infection centers per plot, significantly greater than all other treatments (data not shown). No significant differences were detected in area under the dollar spot progress curve data.

Significant whole plot differences were detected on four of the six rating dates over the course of both years (Table 3.4). Using area under the dollar spot progress curve data, whole plots receiving iprodione + chlorothalonil contained less dollar spot than the non-treated whole plot receiving no fungicide (Table 3.8).

Brown Patch

Brown patch pressure was low (< 3 % in all subplot treatments) throughout the trial period and was only present on a single rating date (data not presented) and did not significantly impact any other measured parameter. No significant overspray-by-fungicide difference was detected on any rating date (Table 3.8).

Cyanobacteria

No significant fungicide or overspray-by-fungicide interactions were detected on any rating date. The overspray whole plot significantly reduced populations of cyanobacteria on all rating dates (Table 3.4), and was also significant for the area under the cyanobacteria progress curve (Table 3.8).These findings are similar to those observed by Elliott (1998) that chlorothalonil is an effective fungicide for reducing the severity of chlorothalonil on putting greens.

CONCLUSION

Repeated applications of strobilurin fungicides did not result in significantly greater NDVI, RVI, turfgrass cover, or visual quality of creeping bentgrass at any time during this two-year field experiment. For each parameter, values for each treatment declined concurrently as the summer progressed each year. Mean spectral reflectance measurements from 14 DAIT to 84 DAIT declined from 0.728 to 0.654 in NDVI and from 6.39 to 4.84 for RVI. Mean turfgrass cover declined by approximately 8% over the course of the trial period and turfgrass quality declined from 6.9 to 6.6 over this same time period. In addition, treatments did not significantly affect visual root length at the termination of the trial period. Significant differences in disease severity were also limited. Dollar spot was significant on a single rating date but yielded no significant effect on any other measured parameter. At no time was brown patch or cyanobacteria severity significantly different between treatments. Our results are similar to prior field studies observing few significant differences in observable plant physiological effects subjected to strobilurin fungicide applications in low disease pressure environments (Swoboda and Pedersen, 2009; Weisz et al. 2011).

Previous laboratory studies have investigated the secondary physiological changes occurring after strobilurin treatment. These physiological changes include the inhibition of ethylene, increased anti-oxidant activity, and hormonal shifts among others (Grossmann and Retzlaff, 1997; Köhle et al., 2002; Jabs et al., 2002). These changes in physiological processes have led to observations of increased rooting, enhanced water deficit stress, and delayed leaf senescence in higher plants in controlled environmental conditions (Grossmann and Retzlaff, 1997; Köhle et al., 2002; Brosnan et al., 2010.). In our study, creeping bentgrass was subjected to environmental stressors in the field including supraoptimal temperatures, ultraviolet light radiation, and moisture stress. Furthermore, to cope with these stressors we administered cultural practices including mowing on alternate days, sand topdressing, aerification, and nutrient and water management. These management practices mimic guidelines implemented by turfgrass managers and remain critical in sustaining a quality creeping bentgrass putting green in the transition zone.

Our results do not suggest that the physiological effects attributed to strobilurin fungicides applications were not occurring in this experiment, but the practical implication is unclear. In this field trial, proper creeping bentgrass management practices coupled with low disease pressure may be a contributing factor for the lack of significant differences among treatments. Applications of strobilurin fungicides may provide a promotion of plant health on creeping bentgrass during summer stress, but the observation of plant health effects may be dependent on a number of factors that are currently unknown.

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APPENDIX

TABLES AND FIGURES

Table 3.1. Visual measurements of turfgrass quality (Pooled across two experimental runs) on a 'Dominant Southern' creeping bentgrass (*Agrostis stolonifera* L.) putting green located in Knoxville, TN, under untreated (UNT) or oversprayed (OS) whole plots with various strobilurin fungicides in 2011 and 2012.

Whole plot†	Fungicide treatment	Rate kg ai ha^{-1}	Visual turfgrass quality					
			Days after initial treatment					
			14	28	42	56	70	84
UNT	Trifloxystrobin	0.38	6.8	6.9	6.7	6.0	6.0	6.4
	Fluoxastrobin	0.55	6.7	6.9	6.9	6.3	6.3	6.5
	Pyraclostrobin (SC)	0.55	6.8	6.9	6.9	6.4	6.4	6.6
	Azoxystrobin (TL)	0.61	6.8	6.9	6.7	6.2	6.2	6.4
	Azoxystrobin (WDG)	0.61	6.7	6.8	6.8	6.2	6.1	6.4
	Pyraclostrobin (WG)	0.55	6.9	6.9	6.8	6.3	6.3	6.7
	Non-treated control		6.8	6.8	6.8	6.1	6.0	6.3
OS	Trifloxystrobin	0.38	6.9	6.9	6.9	6.3	6.3	6.7
	Fluoxastrobin	0.55	7.0	6.9	6.9	6.3	6.2	6.6
	Pyraclostrobin (SC)	0.55	6.9	6.9	6.9	6.2	6.1	6.5
	Azoxystrobin (TL)	0.61	6.9	6.9	6.9	6.4	6.3	6.7
	Azoxystrobin (WDG)	0.61	6.9	6.8	6.9	6.4	6.3	6.7
	Pyraclostrobin (WG)	0.55	6.9	6.9	6.9	6.3	6.3	6.7
	Non-treated control		6.9	6.9	6.9	6.2	6.2	6.7
	$LSD_{0.05}$		NS‡	NS	NS	NS	NS	NS

† Whole plots included an overspray (OS) of a fungicide mixture containing chlorothalonil and iprodione applied every 14 days to control foliar disease at 8.05 and 3.05 kg aI ha^{-1}, respectively, and an untreated treatment (UNT).
‡ NS, not significant.

Table 3.2. Analysis of variance (Pooled across two experimental runs) for turfgrass quality and turfgrass cover data collected on a 'Dominant Southern' creeping bentgrass (*Agrostis stolonifera* L.) putting green subjected to various strobilurin fungicide treatments and overspray combinations in 2011 and 2012.

			Days after initial treatment					
		df	**14**	**28**	**42**	**56**	**70**	**84**
Turfgrass Quality	Replication	3	NS[†]	NS	NS	NS	NS	NS
	Overspray (O)	1	***	NS	***	***	NS	**
	Fungicide (F)	6	NS	NS	NS	*	NS	NS
	O x F	6	NS	NS	NS	NS	NS	NS
Turfgrass Cover (%)	Replication	3	NS	NS	NS	NS	NS	NS
	Overspray (O)	1	NS	*	NS	NS	NS	NS
	Fungicide (F)	6	NS	NS	NS	NS	NS	NS
	O x F	6	NS	NS	NS	NS	NS	NS

* Significant at the $p \leq 0.05$ level.
** Significant at the $p \leq 0.01$ level
*** Significant at the $p \leq 0.001$ level.
† NS, not significant.

Table 3.3. Analysis of area under the (x) progress curve (Pooled across two experimental runs) for turfgrass quality, normalized difference vegetation index (NDVI), ratio vegetation index (RVI), and percent turfgrass cover under untreated (UNT) or oversprayed (OS) whole plots with various strobilurin fungicides in 2011 and 2012 . Treatments were located in Knoxville, TN, on a 'Dominant Southern' creeping bentgrass (*Agrostis stolonifera* L.) putting green.

Whole plot†	Fungicide treatment	Rate kg ai ha^{-1}	Area Under the (X) Progress Curve			
			Turfgrass quality	NDVI	RVI	Turfgrass cover
UNT	Trifloxystrobin	0.38	496.3	50.2	402.4	6887.3
	Fluoxastrobin	0.55	508.7	51.7	429.6	7232.9
	Pyraclostrobin (SC)	0.55	511.3	51.8	432.8	7223.7
	Azoxystrobin (TL)	0.61	500.8	50.9	414.5	7025.0
	Azoxystrobin (WDG)	0.61	498.1	50.6	410.3	7005.4
	Pyraclostrobin (WG)	0.55	509.9	51.1	418.7	7165.0
	Non-treated control		496.3	50.3	405.9	7036.5
OS	Trifloxystrobin	0.38	513.4	50.5	408.1	7075.3
	Fluoxastrobin	0.55	513.5	50.9	418.6	7125.0
	Pyraclostrobin (SC)	0.55	507.9	50.2	404.3	6928.7
	Azoxystrobin (TL)	0.61	513.2	50.5	407.3	7034.5
	Azoxystrobin (WDG)	0.61	514.9	50.9	415.2	7096.2
	Pyraclostrobin (WG)	0.55	513.7	50.5	411.1	7048.6
	Non-treated control		511.1	50.9	415.6	7010.0
	$LSD_{0.05}$		NS‡	NS	NS	NS

† Whole plots included an overspray (OS) of a fungicide mixture containing chlorothalonil and iprodione applied every 14 days to control foliar disease at 8.05 and 3.05 kg ai ha^{-1}, respectively, and an untreated treatment (UNT).
‡ NS, not significant.

Table 3.4. Analysis of variance (Pooled across two experimental runs) for dollar spot, brown patch, and cyanobacteria on a 'Dominant Southern' creeping bentgrass (*Agrostis stolonifera* L.) putting green subjected to various strobilurin fungicide treatments and overspray combinations in 2011 and 2012.

			Days after initial treatment						
		df	**14**	**28**	**42**	**56**	**70**	**84**	**AU(X)PC**
Dollar spot	Replication	3	NS†	NS	NS	NS	NS	NS	NS
	Overspray (O)	1	NS	NS	**	**	**	***	***
	Fungicide (F)	6	NS	NS	NS	NS	NS	NS	NS
	O x F	6	NS	NS	NS	NS	NS	**	NS
Brown patch	Replication	3	NS	NS	NS	NS	NS	NS	NS
	Overspray (O)	1	NS	NS	NS	NS	NS	NS	NS
	Fungicide (F)	6	NS	NS	NS	NS	NS	NS	NS
	O x F	6	NS	NS	NS	NS	NS	NS	NS
Cyano-bacteria	Replication	3	NS	NS	NS	NS	NS	NS	NS
	Overspray (O)	1	***	***	***	**	***	***	***
	Fungicide (F)	6	NS	NS	NS	NS	NS	NS	NS
	O x F	6	NS	NS	NS	NS	NS	NS	NS

** Significant at the $p \leq 0.01$ level.
*** Significant at the $p \leq 0.001$ level.
† NS, not significant.

Table 3.5. Spectral reflectance measurements for normalized difference vegetation index (NDVI) (Pooled across two experimental runs) on a 'Dominant Southern' creeping bentgrass (*Agrostis stolonifera* L.) putting green located in Knoxville,TN, under untreated (UNT) or oversprayed (OS) whole plots with various strobilurin fungicides in 2011 and 2012.

Whole plot†	Fungicide treatment	Rate kg ai ha^{-1}	Normalized Difference Vegetation Index					
			Days after initial treatment					
			14	28	42	56	70	84
UNT	Trifloxystrobin	0.38	0.722	0.679	0.676	0.655	0.661	0.651
	Fluoxastrobin	0.55	0.729	0.691	0.697	0.675	0.686	0.669
	Pyraclostrobin (SC)	0.55	0.730	0.692	0.699	0.678	0.688	0.669
	Azoxystrobin (TL)	0.61	0.726	0.686	0.685	0.662	0.675	0.654
	Azoxystrobin (WDG)	0.61	0.723	0.683	0.685	0.653	0.668	0.653
	Pyraclostrobin (WG)	0.55	0.724	0.685	0.686	0.665	0.675	0.669
	Non-treated control		0.720	0.683	0.684	0.652	0.662	0.648
OS	Trifloxystrobin	0.38	0.726	0.686	0.687	0.654	0.660	0.654
	Fluoxastrobin	0.55	0.734	0.693	0.694	0.658	0.663	0.654
	Pyraclostrobin (SC)	0.55	0.731	0.692	0.690	0.649	0.650	0.642
	Azoxystrobin (TL)	0.61	0.728	0.686	0.688	0.647	0.660	0.644
	Azoxystrobin (WDG)	0.61	0.730	0.692	0.689	0.656	0.668	0.650
	Pyraclostrobin (WG)	0.55	0.732	0.695	0.690	0.647	0.654	0.648
	Non-treated control		0.733	0.692	0.694	0.657	0.664	0.657
	$LSD_{0.05}$		NS†	NS	NS	NS	NS	NS

† Whole plots included an overspray (OS) of a fungicide mixture containing chlorothalonil and iprodione applied every 14 days to control foliar disease at 8.05 and 3.05 kg ai ha^{-1}, respectively, and an untreated treatment (UNT).
† NS, not significant.

Table 3.6. Analysis of variance (Pooled across two experimental runs) for normalized difference vegetation index (NDVI) and ratio vegetation index (RVI) on a 'Dominant Southern' creeping bentgrass (*Agrostis stolonifera L.*) putting green subjected to various strobilurin fungicide treatments and overspray combinations in 2011 and 2012.

			Days after initial treatment					
		df	**14**	**28**	**42**	**56**	**70**	**84**
NDVI	Replication	3	NS[†]	NS	NS	NS	NS	NS
	Overspray (O)	1	*	*	NS	NS	NS	NS
	Fungicide (F)	6	NS	NS	NS	NS	NS	NS
	O x F	6	NS	NS	NS	NS	NS	NS
RVI	Replication	3	NS	NS	NS	NS	NS	NS
	Overspray (O)	1	*	NS	NS	NS	NS	NS
	Fungicide (F)	6	NS	NS	NS	NS	NS	NS
	O x F	6	NS	NS	NS	NS	NS	NS

*Significant at the $p \leq 0.05$ level.
†NS = not significant.

Table 3.7. Analysis of visual root length (Pooled across two experimental runs) on a ‘‘Dominant Southern’ creeping bentgrass (*Agrostis stolonifera* L.) putting green located in Knoxville, TN, under untreated (UNT) or oversprayed (OS) whole plots with various strobilurin fungicides in 2011 and 2012.

Whole plot†	Fungicide treatment	Rate kg ai ha^{-1}	Visual root length (cm)
UNT	Trifloxystrobin	0.38	9.8
	Fluoxastrobin	0.55	10.0
	Pyraclostrobin (SC)	0.55	9.6
	Azoxystrobin (TL)	0.61	9.9
	Azoxystrobin (WDG)	0.61	10.2
	Pyraclostrobin (WG)	0.55	10.2
	Non-treated control		10.1
OS	Trifloxystrobin	0.38	9.6
	Fluoxastrobin	0.55	10.1
	Pyraclostrobin (SC)	0.55	9.6
	Azoxystrobin (TL)	0.61	10.0
	Azoxystrobin (WDG)	0.61	10.0
	Pyraclostrobin (WG)	0.55	9.5
	Non-treated control		9.5
	$LSD_{0.05}$		NS‡

† Whole plots included an overspray (OS) of a fungicide mixture containing chlorothalonil and iprodione applied every 14 days to control foliar disease at 8.05 and 3.05 kg ai ha^{-1}, respectively, and an untreated treatment (UNT).
‡ NS, not significant.

Table 3.8. Analysis of area under the (x) progress curve (Pooled across two experimental runs) for turfgrass quality, normalized difference vegetation Index (NDVI), ratio vegetation index (RVI), turfgrass cover, dollar spot, and cyanobacteria under untreated or over sprayed whole plots in 2011 and 2012. Treatments were located in Knoxville, TN, on a 'Dominant Southern' creeping bentgrass (*Agrostis stolonifera* L.) putting green.

Whole plot†	Area Under the (X) Progress Curve					
	Turfgrass quality	NDVI	RVI	Turfgrass cover	Dollar spot	cyanobacteria
Untreated	503.1	50.9	416.3	7082.3	188.3	59.7
Oversprayed	512.5	50.6	411.4	7045.5	19.0	7.9
$LSD_{0.05}$	NS‡	NS	NS	NS	22.5	10.4

† Whole plots included an overspray (OS) of a fungicide mixture containing chlorothalonil and iprodione applied every 14 days to control foliar disease at 3.05 and 8.05 kg ai ha^{-1}, respectively, and an untreated treatment (UNT).

‡ NS, not significant.

CONCLUSIONS

Experiments were conducted to evaluate the performance of strobilurin fungicides for disease control and their potential to promote physiological effects on creeping bentgrass. Observable differences in disease control and physiological effects varied depending on the environmental conditions in the field.

Differences in physiological effects coincided with differences in disease severity in this research. In a pilot study, strobilurin programs had significantly less disease severity compared to non-treated controls. Strobilurin programs also had significantly greater physiological effects as measured by turfgrass quality, NDVI, and RVI. In a similar study, disease severity was low across all strobilurin programs and the non-treated control. The non-treated control remained similar in all measured parameters compared to the treated plots until brown patch was observed. At this time, differences in disease severity and physiological effects were reported. Differences among treated plots in disease severity or physiological effects were not observed.

Physiological effects of repeated strobilurin applications in absence of visible foliar disease were investigated on a separate creeping bentgrass putting green. Applications of chlorothalonil in combination with iprodione were broadcasted over strobilurin fungicide and non-treated control plots to limit foliar disease activity. Neither significant differences in disease control nor any measured parameter of plant health were observed between strobilurin treatments on any assessment date over the course of two years.

This research suggests that applications of strobilurin fungicides have a more pronounced effect at improving field observable creeping bentgrass health during times of disease activity. This research is beneficial to turfgrass managers maintaining creeping bentgrass putting greens in the transition zone. Strobilurin fungicides can be an excellent disease control option during summer months. However, this research suggests that applications of strobilurin fungicides are not a prerequisite for summer stress management. Rather, turfgrass managers should administer cultural practices including routine mowing, sand topdressing, aerification, and nutrient and water management. These management practices remain central in sustaining creeping bentgrass through prolonged summer stress environments.

VITA

Jesse J Benelli was born on February 11th, 1984 in Bath NY to Donald and Carol Benelli. Raised in Wellsboro, PA, he attended Wellsboro Area High School and graduated in 2002. Jesse attended Mansfield University to pursue a B.A. in psychology. In January of 2004, he transferred to Penn State University to obtain a B.S. in turfgrass science. After graduating in 2007, he was a research technician for Penn State's turfgrass pathology program. He began his M.S. degree in 2010 at the University of Tennessee. Jesse plans to enroll as a Ph.D. student at the University of Tennessee after graduation.