ABSTRACT

BUTLER, ERNEST LEE. Development of Novel Strategies for Control of Spring Dead Spot of Bermudagrass.
(Under the direction of Lane P. Tredway, H. David Shew, and Charles H. Peacock)

Spring dead spot (SDS) is one of the most severe diseases of bermudagrass in Australia, New Zealand, and in the United States where bermudagrass goes into winter dormancy. Several key factors have hampered research on SDS in the past and are likely due to the lack of complete knowledge about the etiology and epidemiology of SDS, lack of knowledge for optimal fungicide efficacy, and the erratic distribution of SDS in the field, which contributes to experimental error. The objectives of this research were to optimize application methods, application timings, and fungicide efficacy, to evaluate rating methods for analysis of SDS incidence, and to identify the causal organism for SDS of bermudagrass in North Carolina. Between 2002 and 2004, we evaluated the efficacy of five application methods, twelve application timings, and four fungicides. No significant differences were detected among application methods; however application in higher volumes of water tended to provide better control. No significant differences were noted among applications timings when compared to the untreated control, but multiple applications starting in August or September tended to provide better control than single or late season applications. Spring applications had no significant effect on SDS incidence or recovery rate. Of the fungicides that were evaluated in this project, fenarimol and propiconazole were most effective, providing from 44 to 89% and 42 to 54% control, respectively. In the spring of 2003, comparison of three different
assessment methods for SDS were evaluated: digital photography (DP), visual estimation (VE), and the point-intersect method (PI). Results of this study indicate that DP is more effective than VE for assessment of SDS incidence. Digital photography consistently produced higher $r^2$ values and lower CV, MSD, and MSE values than VE. Reductions in experimental error translated directly to differences in mean separations used to compare disease incidence in response to fungicide treatments. Digital photography was not consistently more accurate or precise than PI. In total, two-hundred twenty-one isolates of *Ophiophaerella* were collected between 2003 and 2004. Of the isolates collected, *Ophiophaerella korrae* (186 of 221) was the predominant organism isolated from SDS symptomatic bermudagrass. *Ophiophaerella herpotricha* (22 of 221) was detected at low levels in the Charlotte, Raleigh, and Wilmington regions, whereas *O. narmari* was never detected at all in this study.
DEVELOPMENT OF NOVEL STRATEGIES FOR CONTROL OF

SPRING DEAD SPOT OF BERMUDAGRASS

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

Department of Plant Pathology

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APPROVED BY:

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Dedication

I would like to dedicate this thesis to all my family members and friends for their support. I would especially like to dedicate this to my Mom for turning me into the person I am today, for without her guidance I would be lost. I also would like to dedicate this to my lovely wife April for being my best-friend in the world, who has provided me with outstanding love, care, and support. I also would like to dedicate this to my wife for allowing me to escape reality from time to time through my often weekly visits to the banks of the Neuse River for some enjoyable catfishing. Finally, this thesis is ultimately for my wonderful and ever so lovely daughter, Morgan.
Biography

Ernest Lee Butler was born in Greensboro, NC on April 6, 1976 to Michael Ernest Butler and Linda Duggins Butler. In 1994, he graduated from John Motley Morehead High School in Eden, NC as a North Carolina Scholar, Scholar Athlete, and a member of National Honor Society. Upon receiving an Army ROTC scholarship to NC State University, he chose to attend NC State University and major in electrical engineering. After his freshman year, he chose to switch his major to Agronomy with a concentration in turfgrass management after realizing math was not his best subject. As an undergraduate, he worked part-time at MacGregor Downs C.C. in Cary, NC and completed an internship with Greensboro National G.C. as a crew member on the grounds maintenance staff. He graduated from NC State University with a Bachelor of Science degree in the fall of 1998.

After graduation, he went to work for Bland Landscaping Co. Inc. in Apex, NC as a crew foreman in December 1998. He then went to work as the assistant superintendent of Raleigh Golf Association in Raleigh, NC in May 1999. This is where he discovered a newly described disease on bentgrass putting greens. The disease was Dead Spot, caused by Ophiostoma agrostidis. This is where he met Dr. Henry Wetzel, III. He had always had a strong interest in plant diseases thanks to his professor in undergraduate studies, Dr. David Shew and had always thought about attending graduate school. Dr. Wetzel offered him a job within his program and the chance to go to graduate school. He joined Dr. Wetzel as an agricultural research technician in November 1999. He began his graduate studies in plant pathology in spring
2000 on a part-time basis due to being a full-time employee. Dr. Wetzel left the university for another job in January 2001. He was replaced in June 2002 by Dr. Lane Tredway. Mr. Butler’s research project was directed by Dr. Lane Tredway, Dr. David Shew, and Dr. Charles Peacock. He received a masters of science degree in plant pathology on October 22, 2004.

Mr. Butler now resides in Raleigh, NC with his wife, April, and daughter, Morgan.
Acknowledgements

I would like to thank the Dr. Lane Tredway, Dr. David Shew, and Dr. Charles Peacock for their guidance and support throughout this fascinating journey. The expertise and guidance provided by these individuals was absolutely priceless.

I would like to thank all those that have worked with us in the Turfgrass Pathology group; Mac Malloy, Charles Campbell, Brandon Cawthorne, Mike Hrivnak, Josh Scruggs, and Lisa Johnson. Without the help of these outstanding people, this project would have never been completed.

I would like to thank the graduate students in Plant Pathology for all the great times learning together. I would like to personally thank Crop Science graduate students David Lee, Patrick Gregg, Casey Reynolds, and James Rutledge for their friendship, good times, and support throughout this ordeal.

I would like to thank all the staff members in the departments of Plant Pathology and Crop Science for friendship, assistance, and guidance. I would personally like to thank Mike Adams, Greg Parra, Josh McIntyre, Les Privette, Travis Gannon and Jason Hinton for their friendship and support throughout.

Finally, I would like to thank Dr. Henry Wetzel for allowing me the opportunity to pursue this great achievement in my life. Without his willingness to give me a chance at research and grad school, this thesis would likely not exist. I would also like to thank the fungus *Ophiopsphaerella agrostis*, for without its timely arrival in Raleigh, NC, this thesis would likely not exist as well.
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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW
INTRODUCTION

Spring dead spot is a severe patch disease of bermudagrass (*Cynodon* spp.), caused by *Ophiosphaerella herpotricha*, *O. korrae*, and *O. narmari*. The disease was first observed in Oklahoma in 1936 (Smith et al., 1989), but was not officially named spring dead spot until 1960 by Wadsworth & Young (1960). This disease is known to occur in New South Wales, Australia and regions of the United States where bermudagrass goes into winter dormancy (Couch, 1995). It has been recorded in Alabama, Arkansas, California, North Carolina, South Carolina, Georgia, Kansas, Maryland, Mississippi, Missouri, Nebraska, Tennessee, Texas, and Virginia (Couch, 1995). *Ophiosphaerella korrae* is also a pathogen of Kentucky bluegrass (*Poa pratensis* L.), bentgrass (*Agrostis* spp.), creeping red fescue (*Festuca rubra* L.), annual bluegrass (*Poa annua* L.), and rough bluegrass (*Poa trivialis* L.), causing a disease commonly known as necrotic ring spot on these hosts (Smiley & Fowler, 1984; Worf et al., 1986; Smiley et al., 1992).

Bermudagrass is used in North Carolina from the Piedmont to the Coastal Plain in various situations where turf can be grown. It is the primary grass used for high maintenance landscapes and playing surfaces for golf, baseball, football and soccer. Since North Carolina is situated in the transition zone (Turgeon, 1991), growers are faced with the daunting task of growing this warm-season grass in a region that has a cold enough winter season that induces dormancy. In addition to the potential for injury during the winter from cold, freezing, or desiccation, bermudagrass is highly susceptible
to damage from fungal diseases, including spring dead spot (SDS), during the fall and spring.

As with any plant disease, it is imperative that we understand as much as possible about the causal organism and its interactions with the host plant. There are many key factors that are unknown about SDS in North Carolina. For example, the identity of the causal agent in North Carolina is unknown. Since etiology of a disease is critical to understanding its epidemiology, effective control programs have been difficult to predict or recommend. Current cultural recommendations improve the health and growing conditions of bermudagrass and may reduce predisposition to SDS, but they do not provide adequate disease suppression alone. Chemical control recommendations are limited and vague. When chemicals are used, results are highly variable. Therefore, it is easy to understand why SDS control is difficult and frustrating for turfgrass managers. This project is based on the need to answer such critical questions about SDS of bermudagrass.

**LITERATURE REVIEW**

Spring dead spot (SDS) is one of the most severe diseases of bermudagrass in Australia, New Zealand, and in the United States where bermudagrass goes into winter dormancy. Spring dead spot is caused by the ectotrophic, root-infecting fungi

*Ophiosphaerella korrae* Walker and Smith (synonym: *Leptosphaeria korrae* Walker and Smith), *O. herpotricha* (Fr.), and *O. narmari* Walker and Smith (synonym: *L. narmari* Walker and Smith) (Crahay et al., 1988; Endo et al., 1985; Smith, 1971; Tisserat et al., 1989; and Walker & Smith, 1972; Wetzel et al., 1999). *Ophiosphaerella herpotricha* is
the most common causal agent of SDS in Kansas, Oklahoma, and Texas (Tisserat et al. 1989), whereas *O. korrae* is the predominant pathogen in Maryland (Crahay et al., 1988). The causal organism for SDS in North Carolina has been reported to be *Gaeumannomyces graminis* (McCarty & Lucas, 1989).

Spring dead spot usually does not appear until a stand of bermudagrass is more than two years old (Couch, 1995). Symptoms of SDS appear in the spring as bermudagrass resumes growth from winter dormancy. Typical field symptoms are circular patches or rings of brown to straw-colored turf ranging from 15 cm to several meters in diameter. Plants within patches remain dormant and eventually die, collapsing to the ground. Individual patches reappear annually, gradually expand in size, and often coalesce to form larger areas of afflicted turf. The patches may not be easily detected where bermudagrass has been overseeded or in stands with high populations of winter weeds (Couch, 1995). On individual plants, extensive necrosis of stolons, rhizomes, and roots is evident at the time of symptom expression. The majority of infection and colonization is thought to occur in the fall, which may predispose the bermudagrass to winter injury (Lucas, 1980; Lucas & Gilbert, 1979).

Recovery from spring dead spot injury occurs through spread of stolons into the patch from the outside. In severe cases, remnants of patches may still be evident in late summer or early fall. Even if full recovery does occur in these patches, it is likely that the same patches will recur the following season and also increase in size. After 2 to 3 years, turf in the middle of SDS patches can remain alive, thus creating a ring-like symptom. In older stands that are severely afflicted with spring dead spot, patches can
coalesce and the overall damage will appear non-uniform and may be misdiagnosed as winter injury (Smiley et al., 1992).

Diagnosis of SDS is typically performed based on field symptoms and observation of necrotic roots, stolons, and or rhizomes. In order to obtain a definitive diagnosis, one must confirm the presence of a particular pathogen. *Ophiosphaerella* spp. are members of the ectotrophic root-infecting fungi (ERI). Ectotrophic root-infecting fungi colonize roots before penetrating the vascular tissue of the host. Typically, ERI fungi incite necrosis of the roots, stolons and rhizomes of turfgrass host plants. These pathogens cause patch symptoms on many turfgrasses and include species from the genus *Gaeumannomyces, Ophiosphaerella, Leptosphaeria, Magnaporthe,* and *Phialophora* (Wetzel et al., 1996). These fungi produce few distinctive morphological features that can be used for identification. Typically, all of these organisms produce dark brown to black septate hyphae that grow along roots or stolons. Some species produce infection structures, called hyphopodia that may be used for identification. The only way to positively identify many ERI fungi is to induce production of the sexual stage *in vitro.* However, this is very time consuming and often unsuccessful. Another method for identification of ERI fungi would be through culture morphology. Wetzel et al. (1996) showed that *O. korrae* colonies exhibited raised or dome-shaped mycelium and *O. herpotricha* exhibited brownish-black colored exudates in the middle of 2-week-old cultures. Even though this may be effective, it is difficult to definitively identify these organisms on those characters alone.

More recently, it has been shown that *Ophiosphaerella* species can be identified using molecular techniques such RAPD-PCR. Wetzel et al. (1996) used this technique to
identify common ERI fungi such as *Gaeumannomyces* spp., *Magnaportha* sp., *Ophiosphaerella* spp., and *Phialophora* sp. However, RAPD-PCR does not have the specificity of modern PCR techniques and therefore is considered an out-of-date technique.

Wetzel et al. (1999) demonstrated a technique for *Ophiosphaerella* spp. that was accomplished by using PCR primers ITS4 and ITS5, which amplify the ITS1-5.8S-ITS2 region of the rDNA. Tisserat et al. (1994) found that by using universal primers ITS4 and ITS5, *O. herpotricha* isolates always resulted in a 590-bp DNA fragment and *O. korrae* isolates yield either a 590 or 1,019-bp DNA fragment. Based on sequences of the ITS1-5.8S-ITS2 regions, specific PCR primers were developed for *O. korrae* and *O. herpotricha*. *O. herpotricha* primers, OH1 and OH2, amplified a 454-bp fragment from *O. herpotricha* DNA and not from 29 other fungal and bacterial species tested, including *O. korrae*. Primers OK1 and OK2 are specific for *O. korrae*, and amplified a 454-bp fragment was amplified from *O. korrae* DNA and not from 29 other fungal and bacterial species tested, including *O. herpotricha*.

Although the majority of infection by SDS pathogens is thought to occur in the fall, little specific information is available regarding the epidemiology of SDS. Growth studies of *O. korrae* have shown that growth of the fungus in culture occurs from 15 °C to 30 °C, with the optimal growth occurring at 25 °C (Crahay et al., 1988). Smiley et al. (1985) showed that *O. korrae* isolates grew in vitro at 14 °C to 28 °C. However, ‘Tufcote’ bermudagrass inoculated with *O. korrae* was severely damaged at 15 °C. Damage also occurred at 20 °C, but no disease symptoms were noted at 25 °C and 30 °C (Crahay et al., 1988). Bermudagrass roots grow optimally at 35 °C and very slowly at 15
Spring dead spot may be most severe at temperatures below the optimal range for pathogen growth because of increased host susceptibility at low temperatures.

Effective SDS management requires an integrated approach including cultural practices and, when economical, preventative fungicide applications. University turfgrass extension specialists in the United States recommend that growers reduce nitrogen fertilization late in the growing season with an emphasis on using ammonium based products, increase potash application rates late in the season, maintain pH < 6, provide adequate drainage, core aerify, and reduce thatch accumulation (Martin & Hudgins, 2002; Tisserat, 2002; Vincelli, 2002).

Spring dead spot is typically most damaging on intensively managed turfs as compared to low maintenance areas with low fertility regimes (Smiley et al., 1992). Late season applications of nitrogen have been shown to increase disease severity the following spring (Smiley et al., 1992). In Maryland, applications of ammonium sulfate or ammonium chloride supplemented with potassium chloride reduced spring dead spot the following spring by up to 41% and increased spring green up by up to 75% (Dernoeden et al., 1991) this was likely due to a reduction in soil pH. Martin et al. (2001) showed that SDS patch size was greater in all cultivars tested (Mirage, OKS 91-11, & Jackpot) when mown at 3.8 cm versus 1.3 cm. Bermudagrass cultivars also vary widely in their susceptibility to SDS. The varieties Guymon, Midiron, Midlawn, Midfield, Mirage, and Sundevil are resistant to SDS with Midiron being the most resistant (Tisserat, 2002; Martin & Hudgins, 2002). Arizona, Common, Cheyenne, Jackpot, NuMex Sahara, Oasis, Poco Verde, Primavera, Sonesta, Tifton 10, Tifway, Tifgreen, Tropica, Vamont, and Sunturf are considered to be the most susceptible cultivars of
bermudagrass to SDS (Martin & Hudgins, 2002). In general, cultivars with improved cold tolerance also exhibit increased resistance to SDS (Smith et al., 1989).

Few specific recommendations for SDS control with fungicides are available to turfgrass managers. In fact, several extension services do not recommend preventative fungicide applications for spring dead spot control due to erratic results (Tisserat, 2002; Martin & Hudgins, 2002; Vincelli, 2002). Fenarimol, myclobutanil, azoxystrobin, thiophanate-methyl and propiconazole are the only fungicides currently labeled for SDS control. Fenarimol and myclobutanil are the most commonly used fungicides for control of SDS (personal communications). Some turfgrass managers have reported excellent results from preventative applications of these products, whereas others have not. The lack of specific recommendations regarding application rate, timing, or method may be responsible for these inconsistencies.

Field evaluations of fungicides for the control of SDS have dated back to the 1960’s (Lucas, 1979). Most field trials in the past evaluated fungicides that are either no longer available, still experimental compounds, or not labeled for SDS. Products such as nabam, carboxin, chloroneb, maneb, benomyl, chlorothalonil, PCNB, thiram, tebuconazole, diniconazole, fenarimol, propiconazole, thiophanate-methyl have all been previously tested for SDS with variable results among researchers (Dernoeden, 1993).

In North Carolina, Wetzel (2000) tested fungicides for preventative control of SDS in ‘Tifway’ bermudagrass maintained under golf course conditions. Only fenarimol provided significant control of SDS in this study, which reduced SDS incidence by 71 to 85% and significantly increased turfgrass quality. In Oklahoma, Walker et al. (2001) reported no significant reduction in SDS incidence from preventative application of any
fungicides labeled for SDS. In an application timing study conducted in Virginia by Couch et al. (unpublished data, 2002), applications of myclobutanil applied once in August, twice (Aug and Sept), three times (Aug, Sept, and Oct), or four times (Aug, Sept, Oct, and Nov) significantly reduced SDS incidence when compared to the untreated control. Couch et al. also reported azoxystrobin significantly reduced SDS incidence when applied twice (Sept and Oct) when compared to the untreated control. Butler and Tredway (2003) tested fungicides and reported no significant reduction in SDS incidence when compared to the untreated control in a SDS field trial on ‘Tifway’ bermudagrass in North Carolina. The variability of results among researchers indicates the need for further research on SDS management and control. The potential variation in pathogen species may play a role in this.

Field research of SDS management has been hampered in the past due to the irregular distribution of SDS populations in turf stands. Irregular distribution of SDS produces high experimental error when conducting field trials. Also, the methodology for proper and successful field inoculation of SDS is lacking. Potential solutions to this problem are larger plot sizes (≥1.5 m x 3.0 m), experimental designs with a large number of replications, and the use of highly accurate rating methods. Also, it is important to scout potential research areas during the spring when SDS symptoms are evident. This allows researchers to select research areas with uniform SDS distribution. The use of methods that reduce experimental error are absolutely necessary for the proper evaluation of products for control of SDS.

Accurate and precise assessment of SDS incidence is one potential means for reducing experimental error in field research. Traditionally, SDS has been measured in
research plots through visual estimation (VE). The accuracy and precision of this method for SDS evaluation is not known and may contribute to experimental error.

Visual estimation is the most common assessment method in turfgrass research and is a subjective form of analysis (Skogley & Sawyer, 1992). Data sets collected by VE can be highly variable and difficult to reproduce by other research teams (Richardson, 2001). For example, Horst et al. (1983) found high levels of variability among evaluators when 10 trained turfgrass experts visually evaluated the same turfgrass plots for quality and density. In addition, disease incidence is difficult to assess through VE because the human eye is drawn to diseased plants when incidence levels are less than 50% and is drawn to healthy plants when incidence levels are more than 50% (Hebert, 1982). Despite the disadvantages of VE, this continues to be the most common method for assessment of research plots due to the lack of effective and efficient alternatives.

The point-intersect (PI) method has been used to assess canopy coverage in turfgrass (Laycock & Canaway, 1980) and also has been used widely in other areas such as ecology, weed science, and plant pathology (Wheeler et al., 2000). The PI method involves the use of a grid that is placed directly over an entire plot or sections of a plot. The number of intersections overlaying the character of interest (weeds, disease, plant types, etc.) is then divided by the total number of intersects to yield a percentage of the plot surface area exhibiting that character. The density of intersections can be varied depending on the level of accuracy needed. Advantages of PI may include less subjectivity and reduced variability among different evaluators. However, the time and labor required to obtain data through PI can be very demanding and therefore may fall outside a project’s constraints (Richardson et al., 2001).
Due to recent advances in digital cameras and personal computers, digital photography (DP) and digital image analysis represent potential alternatives to VE and PI for evaluation of disease incidence and other parameters. These versatile methods have been used to determine the nitrogen status of corn in the field (Blackmer & White, 1998), the concentration of Brilliant Blue FCF dye on the soil surface (Ewing & Horton, 1999), and the incidence of simulated insect damage on soybean (Glycine max) leaves (O’Neal et al., 2002). In turfgrass research, DP has been used to assess canopy coverage, turfgrass color, and has been shown to be highly accurate and precise in turfgrass systems (Richardson et al., 2001; Karcher et al. 2003). They demonstrated DP provides improved precision, removes bias, and that this process can be accomplished from beginning to end faster than the PI method even by non-turfgrass experts.

Digital photography also has potential applications in assessment of the incidence and severity of plant diseases. Kokko et al. (1993) used DP to assess the severity of common root rot caused by Cochliobolus sativus in wheat (Triticum aestivum L. em Thell.). Compared to VE, DP was rapid, highly precise, and compatible with several image analysis software packages. DP may represent an alternative to VE and PI for assessment of SDS incidence, but has not been evaluated under field conditions.

**RESEARCH OBJECTIVES**

1. **Develop effective and specific recommendations for control of SDS**

   Current recommendations for SDS control using fungicides are vague and often unreliable. Application timing and application method play an important role in the
control of any disease, but little research has been conducted to evaluate the influence of these parameters on SDS control. Field research was conducted from 2002 to 2004 to evaluate products, application methods, and application timings for SDS control in North Carolina. The four fungicides evaluated were: azoxystrobin (Heritage 50WG, 1.22 kg ha\(^{-1}\)), fenarimol (Rubigan 1AS, 18.35 L ha\(^{-1}\)), myclobutanil (Eagle 40WP, 3.67 kg ha\(^{-1}\)), and propiconazole (Banner Maxx 1.4ME, 12.23 L ha\(^{-1}\)). The five application methods that were evaluated were: foliar applications in water equivalent to 0.1, 0.2, or 0.4 L m\(^{-2}\), a watered-in treatment (applied to the foliage in 0.1 L m\(^{-2}\) then irrigated with 6 mm H\(_2\)O immediately after application), or high-pressure soil injection at 2.46x10\(^6\) kgf m\(^{-2}\) using a Cushman Envirojet (Textron Inc., Charlotte, NC). The optimal application timing was determined by evaluating 12 timing regimes for each fungicide. They were either applied August once (A), September once (S), October once (O), November once (N), August monthly (ASON), August monthly with a follow up spring application (+spring) upon bermudgrass green-up (ASON +spring), September monthly (SON), September monthly with a spring application (SON +spring), October monthly (ON), October monthly with a spring application (ON +spring), or November with a spring application (N + spring).

2. **Compare methods for assessment of SDS incidence**

The method used to evaluate treatment effects in science greatly influences our reported results. Therefore, it is highly critical that one chooses a method with the least potential for error and the greatest repeatability. Traditionally, SDS incidence has been evaluated using one of two methods: visual estimation (VE) or point-intersect (PI). Due to recent advances in digital cameras and personal computers, digital photography (DP)
and digital image analysis represent potential alternatives to VE and PI for evaluation of disease incidence and other parameters.

The accuracy, precision, and efficiency of VE, PI, and DP for assessment of SDS incidence in bermudagrass turf was compared by applying each of these three methods to 613 research plots in 4 field trials in 2003.

3. Determine Etiology of SDS in North Carolina

Due to the lack of adequate research on SDS of bermudagrass in North Carolina, several basic questions about SDS need to be answered so that effective management programs can be developed. Determination of the etiology of SDS is the logical first step, because without knowing the etiology, it is not possible to fully understand the epidemiology or management of SDS. This was accomplished by conducting a population survey across five regions of North Carolina in the spring of 2003 and 2004. 196 (2003) and 25 (2004) isolates were collected and identified to the species level using the species specific primers developed by Tisserat et al. (1994) and Wetzel et al. (1999).
LITERATURE CITED


CHAPTER TWO

TIMING & METHOD OF FUNGICIDE APPLICATIONS FOR CONTROL OF SPRING DEAD SPOT IN HYBRID BERMUDAGRASS
INTRODUCTION

Spring dead spot (SDS) is one of the most severe diseases of bermudagrass in Australia, New Zealand, and in the United States where bermudagrass goes into winter dormancy. Spring dead spot is caused by the ectotrophic, root-infecting fungi *Ophiosphaerella korrae* Walker and Smith (synonym: *Leptosphaeria korrae* Walker and Smith), *O. herpotricha* (Fr.), and *O. narmari* Walker and Smith (synonym: *L. narmari* Walker and Smith) (Crahay et al., 1988; Endo et al., 1985; Smith, 1971; Tisserat et al., 1989; and Walker & Smith, 1972; Wetzel et al., 1999). *Ophiosphaerella herpotricha* is the most common causal agent of SDS in Kansas, Oklahoma, and Texas (Tisserat et al. 1989), whereas, *O. korrae* is the predominant pathogen in Maryland (Crahay et al., 1988). The causal organism for SDS in North Carolina has been reported to be *Gaeumannomyces graminis* (McCarty & Lucas, 1989).

Spring dead spot usually does not appear until a stand of bermudagrass is more than two years old (Couch, 1995). Symptoms of SDS appear in the spring as bermudagrass resumes growth from winter dormancy. Typical field symptoms are circular patches or rings of brown to straw-colored turf ranging from 15 cm to several meters in diameter. Plants within patches remain dormant and eventually die, collapsing to the ground. Individual patches reappear annually, gradually expand in size, and often coalesce to form larger areas of afflicted turf. The patches may not be easily detected where bermudagrass has been overseeded or in stands with high populations of winter weeds (Couch, 1995). On individual plants, extensive necrosis of stolons, rhizomes, and roots is evident at the time of symptom expression. The majority of infection and
colonization is thought to occur in the fall, which may predispose the bermudagrass to winter injury (Lucas, 1980; Lucas & Gilbert, 1979).

Although the majority of infection by SDS pathogens is thought to occur in the fall, little specific information is available regarding the epidemiology of SDS. Growth studies of *O. korrae* have shown that growth of the fungus in culture occurs from 15 °C to 30 °C, with optimal growth occurring at 25 °C (Crahay et al., 1988). Smiley et al. (1985) showed that *O. korrae* isolates grew *in vitro* at 14 °C to 28 °C. However, ‘Tufcote’ bermudagrass inoculated with *O. korrae* was severely damaged at 15 °C. Damage also occurred at 20 °C, but no disease symptoms were noted at 25 °C and 30 °C (Crahay et al., 1988). Bermudagrass roots grow optimally at 35 °C and very slowly at 15 °C. Spring dead spot may be most severe at temperatures below the optimal range for pathogen growth because of increased host susceptibility at low temperatures.

Effective SDS management requires an integrated approach including cultural practices and, when economical, preventative fungicide applications. Spring dead spot is typically most damaging on intensively managed turfs as compared to low maintenance areas with low fertility regimes (Smiley et al., 1992). Late season applications of nitrogen have been shown to increase disease severity the following spring (Smiley et al., 1992). In Maryland, applications of ammonium sulfate or ammonium chloride supplemented with potassium chloride reduced spring dead spot the following spring by up to 41% and increased spring green up to 75% (Dernoeden et al., 1991), this was likely due to a reduction in soil pH. Martin et al. (2001) showed that SDS patch size was greater in all cultivars tested (Mirage, OKS 91-11, & Jackpot) when mown at 3.8 cm versus 1.3 cm. Bermudagrass cultivars also vary widely in their susceptibility to SDS. In
general, cultivars with improved cold tolerance also exhibit increased resistance to SDS (Smith et al., 1989).

Few specific recommendations for SDS control with fungicides are available to turfgrass managers. In fact, several extension services do not recommend preventative fungicide applications for spring dead spot control due to erratic results (Tisserat, 2002; Martin & Hudgins, 2002; Vincelli, 2002). Fenarimol, myclobutanil, azoxystrobin, thiophanate-methyl and propiconazole are the only fungicides currently labeled for SDS control. Fenarimol and myclobutanil are the most commonly used fungicides for control of SDS. Some turfgrass managers have reported excellent results from preventative applications of these products, whereas others have not. The lack of specific recommendations regarding application rate, timing, or method may be responsible for these inconsistencies.

Field evaluations of fungicides for the control of SDS have dated back to the 1960’s (Lucas, 1979). Most field trials in the past evaluated fungicides that are either no longer available, still experimental compounds, or not labeled for SDS. Products such as nabam, carboxin, chloroneb, maneb, benomyl, chlorothalonil, PCNB, thiram, tebuconazole, diniconazole, fenarimol, propiconazole, and thiophanate-methyl have been tested previously for control of SDS with variable results among researchers (Dernoeden, 1993).

In North Carolina, Wetzel (2000) tested fungicides for preventative control of SDS in ‘Tifway’ bermudagrass maintained under golf course conditions. Only fenarimol provided significant control of SDS in this study, which reduced SDS incidence by 71 to 85% and significantly increased turfgrass quality. In Oklahoma, Walker et al. (2001)
reported no significant reduction in SDS incidence from preventative application of any fungicides labeled for SDS. In an application timing study conducted in Virginia by Couch et al. (unpublished data, 2002), applications of myclobutanil applied once in August, twice (Aug and Sept), three times (Aug, Sept, and Oct), or four times (Aug, Sept, Oct, and Nov) significantly reduced SDS incidence when compared to the untreated control. Couch et al. also reported azoxystrobin significantly reduced SDS incidence when applied twice (Sept and Oct) when compared to the untreated control. Butler and Tredway (2003) tested fungicides and found no significant reduction in SDS incidence when compared to the untreated control in a SDS field trial on ‘Tifway’ bermudagrass in North Carolina. The variability of results among researchers indicates the need for further research on SDS management and control. The potential variation in pathogen species present may play a role in this.

The objectives of this research are to evaluate the timing and method of fungicide application for control of SDS in hybrid bermudagrass and to evaluate the efficacy of fungicides labeled for SDS control.

**MATERIALS & METHODS**

**Application Methods Study**

Fields of ‘Tifway 419’ bermudagrass at Walnut Creek Softball Complex in Raleigh, NC were scouted in June 2002 while SDS symptoms were evident. These fields are maintained under athletic field conditions and mown at a height of 2.5 cm. Two field experiments were established on Fields #4 and #5 in areas that were severely damaged by
SDS. A split-plot, randomized complete block experimental design was employed, with subplots being 1.5 x 3.0 m in size. On Field #4, treatments were replicated 8 times, the main-plots consisted of fungicides, and the sub-plots were application methods. On Field #5, treatments were replicated 5 times, the main-plots consisted of application method, and the sub-plots were fungicides. Application methods included surface applications (SA) in water equivalent to 0.1, 0.2, or 0.4 L m\(^{-2}\), a watered-in treatment (WI) (applied to the foliage in 0.1 L m\(^{-2}\) then irrigated with 6 mm H\(_2\)O immediately after application), or high-pressure soil injection at 2.21 \times 10^7 \text{ Pa} using a Cushman Envirojet (Textron Inc., Charlotte, NC). The fungicides applied were azoxystrobin (Heritage 50WG, 1.22 kg ha\(^{-1}\)), fenarimol (Rubigan 1AS, 18.35 L ha\(^{-1}\)), myclobutanil (Eagle 40WP, 3.67 kg ha\(^{-1}\)), and propiconazole (Banner Maxx 1.4ME, 12.23 L ha\(^{-1}\)). All treatments were applied on September 30 and October 31, 2002 (Year One) and on October 2 and October 31, 2003 (Year Two). With exception of the high-pressure soil injection method, all treatments were applied at a pressure of 2.76 \times 10^5 \text{ Pa} using a CO\(_2\) powered backpack sprayer with TeeJet 8004, 8008, or 8010 flat fan nozzles (TeeJet, Tifton, GA 31793) using 0.1, 0.2, and 0.4 L m\(^{-2}\), respectively.

**Timing Study**

The timing study component of this project was conducted on Field #4 at Walnut Creek Softball Complex (WC) in Raleigh, NC and on the Fairways Course at Prestonwood C. C. (PW) in Cary, NC. A split-plot, randomized complete block experimental design was utilized with sub-plots being 1.0 m x 1.8 m. Treatments were replicated 8 times (WC) and 4 times (PW), with the fungicides being the main-plot and
the timings being the sub-plots. The fungicides applied were azoxystrobin (Heritage 50WG, 1.22 kg ha\(^{-1}\)), fenarimol (Rubigan 1AS, 18.35 L ha\(^{-1}\)), myclobutanil (Eagle 40WP, 3.67 kg ha\(^{-1}\)), and propiconazole (Banner Maxx 1.4ME, 12.23 L ha\(^{-1}\)). Only fenarimol and myclobutanil were applied at PW due to space limitations. The timing regimes consisted of 12 programs for each fungicide at both sites: August once (A), September once (S), October once (O), November once (N), August monthly (ASON), August monthly with a spring application upon bermudagrass green-up (ASON + spring), September monthly (SON), September monthly with a spring application (SON + spring), October monthly (ON), October monthly with a spring application (ON + spring), November with a spring application (N + spring), or untreated control (UC) (Table 1). All treatments were applied at a pressure of 2.76 \times 10^5 \text{ Pa} using a CO\(_2\) powered backpack sprayer with TeeJet 8008 flat fan nozzles in a water equivalent of 0.4 L m\(^{-2}\).

**Soil Data**

Soil sampling was conducted in June 2004. Samples were sent to the North Carolina Department of Agriculture (NCDA, Raleigh, NC 27699) for analysis. The NCDA division of soil testing uses the Mehlich method for evaluation of soil chemical properties.

Soil temperature data were obtained from the State Climate Office of North Carolina (Raleigh, NC 27695). Soil temperature data was analyzed during the time periods in which application timings tended to be most effective in an attempt to correlate soil temperature data with application timing efficacy.
Assessment Method

Spring dead spot incidence was assessed over two years using digital photography (DP) on May 13, May 27, June 10, and Jun 24, 2003 and on May 13, May 27, and June 9, 2004. Digital photography was conducted by taking a digital image of each plot with a Nikon CoolPix 5700 (Nikon Inc., Melville, NY 11747) digital camera mounted on a custom-made monopod. The monopod was constructed of square aluminum tubing (5-cm wide). The monopod was designed with both vertical and horizontal adjustments so that the camera could be centered directly over plots of varying sizes. For the 1.5 x 3.0 m plots employed in the Application Methods study, the vertical setting was 3.35 m high and the horizontal setting was 1.0 m from the center of the monopod at a 90° angle. For the 1.0 m x 1.8 m plots employed in the Timing Study, the vertical setting was 1.8 m high and the horizontal setting was 0.5 m from the center of the monopod at a 90° angle. The monopod has a 30.5 cm horizontal guide at the base that is parallel to the ground so that the images are taken at the exact same location each time. The camera is attached to the end of the horizontal adjustment using a standard tripod screw so that the camera faces directly towards the ground.

Cotton string was used to mark the borders of all plots for digital photography. The digital camera was set to fully automatic mode; therefore shutter speed and aperture were adjusted according to current light conditions. Images were 2560 x 1920 pixels in size and saved to a 512 MB compact flash card. Photos were taken in the morning hours from 0800 to 1130 hrs and then in the afternoon from 1430 to 1700 hrs to avoid shadows from the monopod being in the image.
Each image was transferred to a personal computer, cropped to remove the pixels outside each plot, resized to 680 x 400 pixels, and then saved in JPEG (joint photographic experts group, .jpg) format. Spring dead spot incidence in each image was measured using SigmaScan Pro v. 5.0 (SPSS, Inc., Chicago, IL 60611). The percentage of green pixels (hue = 35 to 235; saturation = 0 to 100) in each image, corresponding to healthy turf, was measured. SDS incidence was calculated by subtracting the percentage of green pixels from 100 to obtain the percentage of diseased pixels in each plot.

Data from each rating date was subjected to analysis of variance (ANOVA) using SAS 8.02 (SAS Inc., Cary, NC 27513). Separation of mean values for initial disease incidence ($Y_i$) and recovery rate ($r$) were conducted using the Waller-Duncan k-ratio t-test (k=100). Fisher’s protected LSD ($p = 0.05$) was employed for the timing study at Prestonwood CC to separate mean values due to only two fungicide variables. When determining $r$, data were square-root transformed and then subjected to simple linear regression using SAS 8.02 for each plot. All data presented in the application methods study about fungicides are averaged across all application methods and vice-versa. All data presented in the timing study about application timings are averaged across all fungicides and vice-versa.

**RESULTS**

The initial assessment of disease incidence in the spring was used to indicate the degree of preventative control provided by treatments applied the previous fall. Assessment of disease incidence on regular intervals may be conducted for calculation of turf recovery rate in response to fungicide applications or other treatments. In this study,
we focused on both initial and subsequent assessments, because both initial disease and recovery rate are parameters of interest for SDS research.

Symptoms of SDS appeared when the bermudagrass broke dormancy, which was around mid-April in both years. Plots were rated for initial disease control shortly after 100% green-up on 13 May 03 and 12 May 04. Plots were then assessed bi-weekly until SDS symptoms were no longer evident, which was in late June in 2003 and early June in 2004. Plot data from all rating dates were subjected to simple linear regression to determine recovery rate. All data presented about fungicides are averaged across all application methods and vice-versa. Soil test results for all sites can be found in Table 2.

**Application Methods: Field 4 Methods Study**

Experimental design for this study was a split-plot, randomized complete block, with the main-plots being the fungicides and the sub-plots being the application methods.

After applications were made in fall 2002, distinct differences in turf density of the dormant bermudagrass in January 2003 were observed. All fungicides exhibited significantly better winter density when compared to the control, with azoxystrobin and propiconazole resulting in the greatest density (Figure 1). Foliar applications made in 0.1 L m\(^{-2}\), 0.2 L m\(^{-2}\), and 0.4 L m\(^{-2}\) showed significantly better winter density than treatments watered-in or applied with the Envirojet (Figure 2).

In the spring of 2003, plots were rated for green-up on 4 Apr 03. Propiconazole was the only fungicide that significantly reduced green-up when compared to the untreated control (Figure 3); however, azoxystrobin and fenarimol were not significantly different. There were no differences among application methods for green-up (Figure 4).
In 2003, azoxystrobin, fenarimol, and propiconazole significantly reduced disease incidence respectively by 45, 66, and 51% respectively (Figure 5) (ANOVA P-values in Table 3). Myclobutanil provided an intermediate level of control, but was not significantly different from the untreated control. No significant differences were detected among the application methods (Figure 6). However, the WI treatment tended to reduce disease incidence when compared to all other methods. Only fenarimol was significantly slower to recover in the spring when compared to the untreated control (Figure 7). There were no significant differences detected among application methods for $r$ (Figure 8).

In 2004, fenarimol provided the best control of SDS, by reducing disease incidence by 89% (Figure 5) (ANOVA p-values can be found in Table 3). Plots treated with propiconazole exhibited lower SDS incidence compared to azoxystrobin and the untreated control. Myclobutanil provided an intermediate level of control, but was not significantly different from the untreated control. No significant differences were detected among application methods in 2004 (Figure 6). No treatments were significantly faster to recover than the untreated control; however, azoxystrobin was significantly slower to recover than myclobutanil (Figure 9). There were no significant differences detected among application methods for $r$ (Figure 10).

**Application Methods: Field 5 Fungicide Study**

Experimental design for this study was a split-plot, randomized complete block, with the main-plots being the application methods and the sub-plots being the fungicides.
In the spring of 2003, plots were rated for green-up. On this field, plots were rated on 4 Apr 03 and on 21 Apr 03. On the 4 Apr date, propiconazole was significantly slower to green-up when compared to azoxystrobin and myclobutanil (Figure 11). On the 21 Apr date, propiconazole was significantly slower than all treatments (Figure 11). On the 4 Apr date, there were no significant differences among application methods (Figure 12). On the 21 Apr date, Envirojet plots had greened-up significantly more than all other treatments (Figure 12).

In 2003, no significant differences were detected among the application methods (Figure 6) (ANOVA p-values can be found in Table 3). However, treatments SA 0.2 L, SA 0.4 L, and WI tended to reduce disease incidence when compared to SA 0.1 L. No significant differences were detected among the fungicides, however, fenarimol and propiconazole tended to reduce disease incidence (Figure 5). No significant differences were detected among application methods for \( r \) (Figure 13). Fenarimol and propiconazole were significantly slower to recover when compared to the untreated control (Figure 14).

In 2004, no significant differences were detected among the application methods (Figure 6) (ANOVA p-values can be found in Table 3). However, treatments applied in SA 0.2 L, SA 0.4 L, and WI tended to reduce disease incidence when compared to SA 0.1 L. Only fenarimol provided significant suppression of SDS on Field 5 in 2004. Azoxystrobin, myclobutanil, and propiconazole provided an intermediate level of control, but were not significant when compared to the untreated control. No significant differences were detected among application methods (Figure 15) or fungicides (Figure 16) for \( r \).
Timing Study: Walnut Creek Softball Complex

After applications were made in fall 2002, distinct differences in turf density of the dormant bermudagrass were noted in January 2003. Azoxystrobin and propiconazole treatments were significantly denser when compared to plots treated with fenarimol and myclobutanil (Figure 17). All timing treatments exhibited significantly denser turf, except for A, when compared to the untreated control (Figure 18). Multiple applications tended to provide denser turf compared to single applications (Figure 18).

In the spring of 2003, plots were rated for green-up. Azoxystrobin and myclobutanil treated plots greened-up more quickly than fenarimol and propiconazole treated plots (Figure 19). There were no significant differences at green-up among application timings, however, all timings tended to be slower to green-up compared to the untreated control (Figure 20).

In 2003, few significant differences were observed among the 12 application timing treatments, but some trends can be noted (Figure 21) (ANOVA P-values in Table 4). A single application made in A, S, or O was more effective than a single application made in N, and multiple applications were more effective than the single applications. Spring applications did not affect control of SDS (Figure 21). According to soil temperature data, fungicide applications were most effective when average daily soil temperatures were between 15 ºC and 30 ºC. All fungicides provided significantly better control of SDS compared to propiconazole (Figure 22).

In 2004, applications made in SON, ASON, and ASON + spring were significantly different from the untreated control (Figure 23) (ANOVA p-values can be
found in Table 4). Spring applications did not affect control of SDS (Figure 23).

According to soil temperature data, fungicide applications were most effective if applied when average daily soil temperatures were between 15 ºC and 30 ºC. Fenarimol provided significantly better control than all other fungicides, with propiconazole providing significantly better control than azoxystrobin and myclobutanil (Figure 23).

**Timing Study: Prestonwood CC**

Spring dead spot incidence was not uniform throughout the field plot in 2003 and in 2004. This contributed to experimental error, and therefore lowered our ability to detect significant differences among treatments.

After applications were made in fall 2002, distinct differences were noted in turf density of the dormant bermudagrass in January 2003. There were no significant differences among fungicide treatments at this site (Figure 24). All multiple application timings except for N + spring and ON + spring had greened-up more quickly than the untreated control (Figure 25).

In 2003, no significant differences were detected among treatments, however, applications in ASON or ASON + spring tended to reduce disease incidence when compared to all other application timings (Figure 26) (ANOVA P-values in Table 4). According to Fishers-protected LSD, fenarimol provided significantly better control than myclobutanil (Figure 27).

In 2004, no significant differences among application timings were detected, but applications in S, SON, and ASON + spring tended to provide better control than all other timings, however, SON + spring and ASON did not exhibit this same trend,
indicating that these differences may be due to experimental error (Figure 28) (ANOVA P-values in Table 4). According to Fishers-protected LSD, no significant differences were noted among the two fungicides treatments (Figure 27).

**DISCUSSION**

Spring dead spot can be managed with an integrated approach, implemented over a period of several years. Improving soil conditions, proper nitrogen fertilization, fall potassium applications, and reduction of soil pH are effective options for reducing SDS incidence. Preventative fungicide applications are an option in high value turf or where cultural practices alone do not provide adequate control. Of the fungicides that were evaluated in this project, fenarimol and propiconazole appear to be most effective in suppressing SDS development providing from 44 to 89% and 42 to 54% control respectively. Propiconazole, however, delayed spring green-up in 2003 at all of the sites in which it was tested. Propiconazole tended to provide much denser turf in the winter, which may be responsible for slower spring green-up. This could possibly be due to inhibition of sunlight by the thicker canopy and/or delaying an increase of soil temperatures.

No significant differences were detected among application methods in this study, but applications in higher volumes of water or when watered-in tended to provide better control compared to the standard application of 0.1 L m\(^2\) or use of the Envirojet. Since *O. korrae* attacks the below-ground tissues of bermudagrass, application methods that deliver the fungicide closer to the causal organism are expected to be most effective. However the Envirojet also placed the fungicide in the root zone and it did not
significantly improve SDS control. The Envirojet delivers product on 5 cm spacings, therefore it is possible that this is too far apart and the chemical is not applied uniformly enough. Applications applied in lower water volumes tended to increase the density of the turf in the winter. Applications in lower water volumes do not place the chemical deep enough into the canopy, therefore protecting the upper portion of the turf plant. As bermudagrass enters winter dormancy, metabolism slows and therefore may retain the fungicide throughout the winter and plant tissue is not being removed by mowing. The retention of fungicide could be inhibiting secondary pathogens from promoting natural senescence of the leaf tissue, therefore creating a denser turf stand in the winter. The Envirojet did encourage a faster spring green-up at one of the two sites tested. It is possible that the aerification effect of the application method increased overall turf health and therefore encouraged spring green-up.

When recovery rates were analyzed, a square-root transformation our original data sets was used. Untreated control plots tended to have significantly more SDS incidence and therefore would have more and larger patches. Spring dead spot patches are typically circular in nature; therefore plots with more and larger patches could potentially have faster recovery rates than those with few or no patches. We used a square-root transformation to linearize the data, and the transformation helped in data interpretation, but more in-depth research on this topic is needed to improve confidence in fungicide recommendations. Results from this study did indicate that the untreated control plots tended to recover quicker in the spring compared to all other fungicides except for myclobutanil.
The precise timing of fungicide applications does not appear to be critical, as long as they are made before soil temperatures fall below 15 °C. This would correlate with the optimal temperatures for pathogen growth, which range from 14 to 30 °C. Also at this temperature, bermudagrass is still active, therefore allowing the plant to absorb the fungicides. Also, control of SDS tended to be better when multiple applications were made starting in August or September. Multiple applications did increase turf density in the winter and tended to increase spring green-up.

Based on the results of this project, control of SDS with fungicides is possible. Unlike some diseases, SDS typically cannot be totally eradicated in one year. The use of the proper fungicide, application method, and application timing along with proper cultural practices over several years will ensure a more satisfactory level of SDS control.

The observations made in this study have helped to clarify areas of interest about SDS that previously were vague. Future research should include repeating the evaluation of different application methods, fungicides, and alternate timings at additional sites. Although, there is not much room for the evaluation of different application methods, similar ones can be evaluated for repeatability. Fungicide testing could involve the same compounds as tested in this study or the evaluation of similar fungicides that are based on the same chemistry or mode of action. Timing studies could be more robust, evaluate timing schemes that are more in-depth and look closer at timings in August through October for climates similar to North Carolina. Finally, of interest, future research on higher profile bermudagrass turf such as golf course greens should be conducted.
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LITERATURE CITED


Figure 1. Effect of fungicides, averaged across all application methods, applied in the fall of 2002 on winter density of bermudagrass on Field 4 at Walnut Creek Softball Complex, Raleigh NC in January 2003.

Values are means of eight replicates. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100). Density measured on a scale of 1-9, with 5 being acceptable and 9 being the best.
Figure 2. Effect of application method, averaged across all fungicides, in the fall of 2002 on winter density of bermudagrass on Field 4 at Walnut Creek Softball Complex, Raleigh NC in January 2003.

Values are means of eight replicates. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100). Density measured on a scale of 1-9, with 5 being acceptable and 9 being the best.
Figure 3. Effect of fungicides, averaged across all application methods, applied in fall 2002 on spring green-up effect of bermudagrass on Field 4 at Walnut Creek Softball Complex in Raleigh, NC on 04 April 2003.

Values are means of eight replicates. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100). Green-up is a rating from 1-9, where 9 is 100% greened-up bermudagrass plot.
Figure 4. Comparison of application methods, averaged across all fungicides, in fall 2002 on spring green-up effect of bermudagrass on Field 5 at Walnut Creek Softball Complex in Raleigh, NC on 04 April 2003.

Values are means of five replicates. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100). Green-up is a rating from 1-9, where 9 is 100% greened-up bermudagrass plot.
Figure 5. Spring dead spot incidence on Fields 4 and 5 at Walnut Creek Softball Complex in Raleigh, NC in 2003 and 2004, averaged across all application methods.

Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).
Figure 6. Effect of application method on SDS incidence on Fields 4 and 5 at Walnut Creek Softball Complex in Raleigh, NC in 2003 and 2004, averaged across all fungicides.

Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).
Figure 7. Recovery rates for fungicides, averaged across all application methods, applied on Field 4 at Walnut Creek Softball Complex in Raleigh, NC in 2003.

Recovery Rate

Fungicide

Values are recovery rates based on linear regression of square-root transformed disease incidence values over time. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).
**Figure 8.** Recovery rates for application methods, averaged across all fungicides, on Field 4 at Walnut Creek Softball Complex, Raleigh NC in 2003.

\[^2\text{Values are recovery rates based on linear regression of square-root transformed disease incidence values over time. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).}\]
Figure 9. Recovery rates for fungicides, averaged across all application methods, applied on Field 4 at Walnut Creek Softball Complex in Raleigh, NC in 2004.

Values are recovery rates based on linear regression of square-root transformed disease incidence values over time. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).
Figure 10. Recovery rates for application methods, averaged across all fungicides, on Field 4 at Walnut Creek Softball Complex, Raleigh NC in 2004.

Values are recovery rates based on linear regression of square-root transformed disease incidence values over time. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).
Figure 11. Comparison of fungicides, averaged across all application methods, applied in fall 2002 on spring green-up effect of bermudagrass on Field 5 at Walnut Creek Softball Complex in Raleigh, NC in Apr 2003.

*Values are means of five replicates. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100). Green-up is a rating from 1-9, where 9 is 100% greened-up bermudagrass plot.*
Figure 12. Comparison of application methods, averaged across all fungicides, in fall 2002 on spring green-up effect of bermudagrass on Field 5 at Walnut Creek Softball Complex in Raleigh, NC in April 2003.

*Values are means of five replicates. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100). Green-up is a rating from 1-9, where 9 is 100% greened-up bermudagrass plot.
Figure 13. Recovery rates for application methods, averaged across all fungicides, on Field 5 at Walnut Creek Softball Complex in Raleigh, NC in 2003.

\[ \text{Recovery Rate}^2 \]

<table>
<thead>
<tr>
<th>Application Method</th>
<th>Recovery Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA 0.1 L</td>
<td>a</td>
</tr>
<tr>
<td>SA 0.2 L</td>
<td>a</td>
</tr>
<tr>
<td>SA 0.4 L</td>
<td>a</td>
</tr>
<tr>
<td>VRI</td>
<td>a</td>
</tr>
<tr>
<td>Envirojet</td>
<td>a</td>
</tr>
</tbody>
</table>

Values are recovery rates based on linear regression of square-root transformed disease incidence values over time. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).
Figure 14. Recovery rates for fungicides, averaged across all application methods, applied on Field 5 at Walnut Creek Softball Complex in Raleigh, NC in 2003.

Values are recovery rates based on linear regression of square-root transformed disease incidence values over time. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).
Figure 15. Recovery rates for application methods, averaged across all fungicides, on Field 5 at Walnut Creek Softball Complex in Raleigh, NC in 2004.

Values are recovery rates based on linear regression of square-root transformed disease incidence values over time. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).
Figure 16. Recovery rates for fungicides, averaged across all application methods, applied on Field 5 at Walnut Creek Softball Complex in Raleigh, NC in 2004.

\[\text{Recovery Rate}^2\]

\[
\begin{array}{c}
\text{Fungicide} \\
\text{aconitrodin} & \text{fenamid} & \text{myclobutanil} & \text{propiconazole} & \text{untreated} \\
\text{a} & \text{a} & \text{a} & \text{a} & \text{a}
\end{array}
\]

Values are recovery rates based on linear regression of square-root transformed disease incidence values over time. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test ($k=100$).
Figure 17. Effect of fungicide, averaged across all timings, applied in the fall of 2002 on winter density of bermudagrass on Field 4 at Walnut Creek Softball Complex, Raleigh NC in January 2003.

Values are means of eight replicates. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).
Density measured on a scale of 1-9, with 9 being acceptable and 0 being the best.
Figure 18. Effect of application timing, averaged across all fungicides, applied in the fall of 2002 on winter density of bermudagrass on Field 4 at Walnut Creek Softball Complex, Raleigh NC in January 2003.

Values are means of four replicates. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100). Density measured on a scale of 1-9, with 5 being acceptable and 9 being the best.
Figure 19. Comparison of fungicides, averaged across all timings, applied in fall 2002 on spring green-up effect of bermudagrass on Field 4 at Walnut Creek Softball Complex in Raleigh, NC on 04 Apr 2003.

\[ \text{Green-up}\]²

²Values are means of eight replicates. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100). Green-up is a rating from 1-9, where 9 is 100% greened-up bermudagrass plot.
Figure 20. Comparison of application timings, averaged across all fungicides, applied in fall 2002 on spring green-up effect of bermudagrass on Field 4 at Walnut Creek Softball Complex in Raleigh, NC on 04 Apr 2003.

Values are means of four replicates. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100). Green-up is a rating from 1-9, where 9 is 100% greened-up bermudagrass plot.
Figure 21. Effect of application timing, averaged across all fungicides, on SDS incidence on Field 4 at Walnut Creek Softball Complex in Raleigh, NC in 2003.

Values are means of four replicates. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).
Figure 22. Effect of fungicides, averaged across all timings, on SDS incidence on Field 4 at Walnut Creek Softball Complex in Raleigh, NC.

![Graph showing disease incidence (%)]

Values are means of four replicates. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).
Figure 23. Effect of application timing, averaged across all fungicides, on SDS incidence on Field 4 at Walnut Creek Softball Complex in Raleigh, NC in 2004.

Values are means of four replicates. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).
Figure 24. Effect of fungicide, averaged across all timings, applied in the fall of 2002 on winter density of bermudagrass at Prestonwood C. C. Cary, NC in January 2003.

![Graph showing the effect of fungicides on bermudagrass density](image)

Values are means of four replicates. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100). Density measured on a scale of 1-9, with 5 being acceptable and 9 being the best.
Figure 25. Effect of application timing, averaged across both fungicides, applied in the fall of 2002 on winter density of bermudagrass at Prestonwood C. C. Cary, NC in January 2003.

Values are means of four replicates. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100). Density measured on a scale of 1-9, with 5 being acceptable and 9 being the best.
**Figure 26.** Effect of application timing, averaged across both fungicides, on SDS incidence at Prestonwood C. C. Cary, NC in 2003.

\[\text{Disease Incidence (\%)} \]

Values are means of four replicates. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).

\[a^2\]
Figure 27. Effect of fungicide, averaged across all timings, on SDS incidence at Prestonwood C. C. Cary, NC.

\[\text{Disease Incidence (\%)}^2\]

Year

<table>
<thead>
<tr>
<th>Year</th>
<th>Disease Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>5</td>
</tr>
<tr>
<td>2004</td>
<td>4</td>
</tr>
</tbody>
</table>

\[\text{Values are means of four replicates. Bars followed by the same letter are not significantly different according to Fisher’s protected LSD (p<0.05).}\]
Figure 28. Effect of application timing, averaged across both fungicides, on SDS incidence at Prestonwood C. C. Cary, NC in 2004.

Values are means of four replicates. Bars followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).
Table 1. Application dates for timing study in 2002 and 2003.

<table>
<thead>
<tr>
<th>Application Timing</th>
<th>Walnut Creek 2002 Application Date</th>
<th>Walnut Creek 2003 Application Date</th>
<th>Prestonwood CC 2002 Application Date</th>
<th>Prestonwood CC 2003 Application Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>August</td>
<td>16 Aug 02</td>
<td>18 Aug 03</td>
<td>16 Aug 02</td>
<td>18 Aug 03</td>
</tr>
<tr>
<td>September</td>
<td>24 Sept 02</td>
<td>25 Sept 03</td>
<td>17 Sept 02</td>
<td>25 Sept 03</td>
</tr>
<tr>
<td>October</td>
<td>18 Oct 02</td>
<td>16 Oct 03</td>
<td>18 Oct 02</td>
<td>16 Oct 03</td>
</tr>
<tr>
<td>November</td>
<td>14 Nov 02</td>
<td>14 Nov 03</td>
<td>14 Nov 02</td>
<td>14 Nov 03</td>
</tr>
<tr>
<td>Spring Application</td>
<td>27 May 03</td>
<td>12 May 04</td>
<td>27 May 03</td>
<td>12 May 04</td>
</tr>
</tbody>
</table>
Table 2. Soil test results from research sites in June 2004.

<table>
<thead>
<tr>
<th>Field Site</th>
<th>pH</th>
<th>CEC</th>
<th>BS%</th>
<th>HM%</th>
<th>Ca %</th>
<th>Mg %</th>
<th>Na</th>
<th>P Index</th>
<th>K Index</th>
<th>Mn Index</th>
<th>Zn Index</th>
<th>Cu Index</th>
<th>S Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walnut Creek Field 4</td>
<td>6.00</td>
<td>6.50</td>
<td>91.00</td>
<td>0.71</td>
<td>62.00</td>
<td>19.00</td>
<td>0.10</td>
<td>69 (h)</td>
<td>119 (vh)</td>
<td>192 (vh)</td>
<td>253 (vh)</td>
<td>231 (vh)</td>
<td>61 (h)</td>
</tr>
<tr>
<td>Walnut Creek Field 5</td>
<td>5.40</td>
<td>5.80</td>
<td>78.00</td>
<td>0.71</td>
<td>52.00</td>
<td>15.00</td>
<td>0.10</td>
<td>74 (h)</td>
<td>126 (vh)</td>
<td>158 (vh)</td>
<td>254 (vh)</td>
<td>229 (vh)</td>
<td>68 (h)</td>
</tr>
<tr>
<td>Prestonwood CC</td>
<td>4.90</td>
<td>6.30</td>
<td>78.00</td>
<td>0.60</td>
<td>50.00</td>
<td>23.00</td>
<td>0.10</td>
<td>30 (m)</td>
<td>71 (h)</td>
<td>494 (vh)</td>
<td>159 (vh)</td>
<td>145 (vh)</td>
<td>48 (m)</td>
</tr>
</tbody>
</table>

Soil test index level as determined by the NCDA Soil Testing Division; m=medium, h=high, vh=very high.
Table 3. ANOVA table p-values for initial rating date of SDS incidence in application methods study at each field site.

<table>
<thead>
<tr>
<th>Field Study</th>
<th>Year</th>
<th>Fungicide</th>
<th>Application Method</th>
<th>Fungicide x Application Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field 4 Methods</td>
<td>2003</td>
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<td>0.8028</td>
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<tr>
<td></td>
<td>2004</td>
<td>&lt; 0.0001</td>
<td>0.4219</td>
<td>0.3705</td>
</tr>
<tr>
<td>Field 5 Fungicides</td>
<td>2003</td>
<td>0.0018</td>
<td>0.0200</td>
<td>0.9844</td>
</tr>
<tr>
<td></td>
<td>2004</td>
<td>&lt; 0.0001</td>
<td>0.5549</td>
<td>0.9510</td>
</tr>
</tbody>
</table>
Table 4. ANOVA table p-values for initial rating date of SDS incidence in application timing study at each field site.

<table>
<thead>
<tr>
<th>Field Site</th>
<th>Year</th>
<th>Fungicide</th>
<th>Timing</th>
<th>Fungicide x Timing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walnut Creek</td>
<td>2003</td>
<td>0.0056</td>
<td>0.0121</td>
<td>0.9688</td>
</tr>
<tr>
<td></td>
<td>2004</td>
<td>&lt; 0.0001</td>
<td>0.0059</td>
<td>0.3576</td>
</tr>
<tr>
<td>Prestonwood CC</td>
<td>2003</td>
<td>0.0081</td>
<td>0.8504</td>
<td>0.2307</td>
</tr>
<tr>
<td></td>
<td>2004</td>
<td>0.0680</td>
<td>0.7482</td>
<td>0.8475</td>
</tr>
</tbody>
</table>
CHAPTER THREE

COMPARISON OF METHODS FOR EVALUATION OF SPRING DEAD SPOT INCIDENCE IN HYBRID BERMUDAGRASS
INTRODUCTION

Spring dead spot (SDS) is one of the most severe diseases of bermudagrass (Cynodon dactylon (L.) Pers. and C. dactylon x C. transvaalensis Burtt-Davy) in Australia, New Zealand, and throughout the United States wherever bermudagrass undergoes winter dormancy. Spring dead spot is caused by the ectotrophic, root-infecting fungi Ophiosphaerella herpotricha, O. korrae Walker & Smith (synonym Leptosphaeria korrae Walker & Smith), and O. narmari Walker & Smith (synonym L. narmari Walker & Smith) (Crahay et al., 1988; Endo et al., 1985; Smith, 1971; Tisserat et al., 1989; and Walker & Smith, 1972; Wetzel et al., 1999). Ophiosphaerella herpotricha is the most common causal agent of SDS in Kansas, Oklahoma, and Texas (Tisserat et al. 1989), whereas O. korrae is the predominant pathogen in Maryland (Crahay et al., 1988) and North Carolina (manuscript in preparation).

Symptoms of SDS appear in spring as bermudagrass resumes growth from winter dormancy. Typical field symptoms are circular patches or rings of brown to straw-colored turf ranging from a few centimeters to several meters in diameter. Individual patches reappear annually, gradually expand in size, and often coalesce to form larger areas of afflicted turf. On individual plants, extensive necrosis of stolons, rhizomes, and roots is evident on individual plants at the time of symptom expression. The majority of infection and colonization is thought to occur in the fall, which may predispose bermudagrass to winter injury (Lucas, 1980; Lucas and Gilbert, 1979).

Effective SDS management requires an integrated approach including cultural practices and, where economical, preventative fungicide applications. However, few specific recommendations for SDS management are available to turfgrass managers. In
fact, several extension services do not recommend preventative fungicide applications for spring dead spot control due to erratic results (Tisserat, 2004; Martin and Hudgins, 2002; and Vincelli, 1998). Historically, research of SDS and its management has been hampered by the erratic distribution of the disease in the field. This often leads to high experimental error and low power for detecting significant differences among treatments. Reducing experimental error, therefore, is critical to the success of SDS research in the field.

Methods for reducing experimental error in SDS research include selection of sites that are uniformly infested with the disease and use of experimental designs includes large plots (1.5 x 3 m) and 6 to 8 replications. Accurate and precise assessment of SDS incidence represents another potential means of reducing experimental error in field research. Traditionally, SDS has been measured in research plots through visual estimation (VE). The accuracy and precision of this method for SDS evaluation is not known and may contribute to experimental error.

Visual estimation is the most common assessment method in turfgrass research and is a subjective form of analysis (Skogley and Sawyer, 1992). Data sets collected by VE can be highly variable and difficult to reproduce by other research teams (Richardson, 2001). Horst et al. (1983) found high levels of variability among evaluators when 10 trained turfgrass experts visually evaluated the same turfgrass plots for quality and density. In addition, disease incidence is difficult to assess through VE because the human eye is drawn to diseased plants when incidence levels are less than 50% and is drawn to healthy plants when incidence levels are more than 50% (Hebert, 1982). Despite the disadvantages of VE, this continues to be the most common method used for
the assessment of treatments in research plots due to the lack of effective and efficient alternatives.

The point-intersect (PI) method has been used to assess canopy coverage in turfgrass (Laycock and Canaway, 1980) and has also been used widely in ecology, weed science, and plant pathology (Wheeler et al., 2000). The PI method involves the use of a grid placed directly over an entire plot or sections of a plot. The number of intersections overlaying the character of interest (weeds, disease, plant types, etc.) is then divided by the total number of intersects to yield a percentage of the plot surface area exhibiting that character. The density of intersections is varied depending on the level of accuracy needed. Advantages of PI include less subjectivity and reduced variability among evaluators. However, the time and labor required to obtain data through PI can be very demanding and therefore may fall outside a project’s constraints (Richardson et al., 2001).

Due to recent advances in digital cameras and personal computers, digital photography (DP) and digital image analysis represent potential alternatives to VE and PI for evaluation of disease incidence and other parameters. These versatile methods have been used to determine the nitrogen status of corn in the field (Blackmer and White, 1998), the concentration of Brilliant Blue FCF dye on the soil surface (Ewing and Horton, 1999), and the incidence of simulated insect damage on soybean (Glycine max) leaves (O’Neal et al., 2002). In turfgrass research, DP has been used to assess canopy coverage, turfgrass color, and has been shown to be highly accurate and precise in turfgrass systems (Richardson et al., 2001; Karcher et al. 2003). They demonstrated DP
provides improved precision, removes bias, and that this process can be accomplished from beginning to end faster than the PI method even by non-turfgrass experts.

Digital photography also has potential applications in assessment of the incidence and severity of plant diseases. Kokko et al. (1993) used DP to assess the severity of common root rot caused by *Cochliobolus sativus* in wheat (*Triticum aestivum* L. em Thell.). Compared to VE, DP was rapid, highly precise, and compatible with several image analysis software packages.

Richardson et al. (2001) compared VE, PI, and DP for assessment of turfgrass cover under controlled conditions. Digital photography may represent an alternative to VE and PI for assessment of SDS incidence, but has not been evaluated under field conditions. The objective of this study was to compare the accuracy, precision, and efficiency of VE, PI, and DP for assessment of SDS incidence in bermudagrass turf and to determine the effect of rating method on experimental errors in field experiments.

**MATERIALS & METHODS**

Assessment methods were implemented in field experiments designed to evaluate the effect of fungicides and application methods on SDS incidence. Fields of ‘Tifway 419’ bermudagrass at Walnut Creek Softball Complex in Raleigh, NC were scouted in June 2002 while SDS symptoms were evident. Two field experiments were established on Fields #4 and #5 in areas that were severely damaged by SDS. A split-plot, randomized complete block experimental design was employed, with subplots being 1.5 x 3 m in size. On Field #4, treatments were replicated 8 times, the main-plots consisted of fungicides, and the sub-plots were fungicide application methods. On Field #5, treatments
were replicated 5 times, the main-plots consisted of applications, and the sub-plots were fungicides. Application methods included foliar applications in water equivalent to 0.1, 0.2, or 0.4 L m⁻², a watered-in treatment (applied to the foliage in 0.1 L m⁻² then irrigated with 6 mm H₂O immediately after application), or high-pressure soil injection at 2.46x10⁶ kgf m⁻² using a Cushman Envirojet (Textron Inc., Charlotte, NC). The fungicides applied were azoxystrobin (Heritage 50WG, 1.22 kg ha⁻¹), fenarimol (Rubigan 1AS, 18.35 L ha⁻¹), myclobutanil (Eagle 40WP, 3.67 kg ha⁻¹), and propiconazole (Banner Maxx 1.4ME, 12.23 L ha⁻¹). All treatments were applied on September 30 and October 31, 2002.

Spring dead spot incidence was assessed using VE, PI, and DP on May 13, May 27, June 10, and Jun 24, 2003. Visual estimation was conducted as described by Tredway et al (2001). In this method, an imaginary grid is visualized over the plot surface and used to estimate the percentage of plot surface area exhibiting disease symptoms. This method was conducted by the same person on all rating dates. For the PI method, a 1.5 x 1.5 m frame was constructed using polyvinylchloride pipe (5-cm diam.). Plastic string (0.24-cm diam.) was woven through the frame on 10.16-cm centers to create a grid with 196 intersects. The grid was laid over the bottom half of the plot, and the number of intersections overlaying SDS symptoms were counted. This process was repeated for the top half of each plot. SDS incidence was calculated by dividing the total number of intersects overlaying diseased turf by 392 (twice the total number of intersects in the grid).

Digital photography was conducted by taking a digital image of each plot with a Nikon CoolPix 5700 (Nikon Inc., Melville, NY 11747) digital camera mounted on a custom-made monopod. The monopod was constructed of square aluminum tubing (5-cm
The monopod was designed with both vertical and horizontal adjustments so that the camera could be centered directly over plots of varying sizes. For the 1.5 x 3.0 m plots employed in this study, the vertical setting was 3.35 m high and the horizontal setting was 1.0 m from the center of the monopod at a 90° angle. The monopod has a 30.5 cm horizontal guide at the base that is parallel to the ground so that the images are taken at the exact same location each time. The camera is attached to the end of the horizontal adjustment using a standard tripod screw so that the camera faces directly towards the ground.

Cotton string was used to mark the borders of all plots for digital photography. The digital camera was set to fully automatic mode; therefore shutter speed and aperture were adjusted according to current light conditions. Images were 2560 x 1920 pixels in size and saved to a 512 MB compact flash card. Photos were taken in the morning hours from 0800 to 1130 hrs and then from 1430 to 1700 hrs to avoid shadows from the monopod being in the image.

Each image was transferred to a personal computer, cropped to remove the pixels outside each plot, resized to 680 x 400 pixels, and then saved in JPEG (joint photographic experts group, .jpg) format. Spring dead spot incidence in each image was measured using SigmaScan Pro v. 5.0 (SPSS, Inc., Chicago, IL 60611). The percentage of green pixels (hue = 35 to 235; saturation = 0 to 100) in each image, corresponding to healthy turf, was measured. Spring dead spot incidence was calculated by subtracting the percentage of green pixels from 100 to obtain the percentage of diseased pixels in each plot.
Within dates, data from each assessment method was subjected to analysis of variance (ANOVA) using SAS 8.02 (SAS Inc., Cary, NC). Comparisons of assessment methods were made based on $r^2$ values, coefficients of variation (CV), and MSD values according to Waller-Duncan k-ratio t-test ($k=100$). Analysis of variance was performed on natural log of the error mean square (MSE) values from each assessment method using assessment dates as replicates. Separation of mean MSE values for each assessment method was conducted using the Waller-Duncan k-ratio t-test ($k=100$). The variance of VE and PI relative to DP was calculated for each assessment date by dividing $MSE_{VE}$ or $MSE_{PI}$ by $MSE_{DP}$. Linear regression was performed using SAS 8.02 to describe the relationships among VE, PI, and DP over varying levels of SDS incidence.

**RESULTS**

For SDS research, the initial assessment of disease incidence in the spring indicates the degree of preventative control provided by treatments applied the previous fall. Assessment of disease incidence at regular intervals was conducted for calculation of turf recovery rate in response to fungicide applications or other treatments. In this study, we focused on both initial and subsequent assessments, because both initial disease and recovery rate are parameters of interest for SDS research.

**Field #4 ‘Methods Study’**

Several statistical parameters were used to compare the experimental error associated with each assessment method (Table 1). $R^2$ values ranged from 0.53 to 0.70 for VE, from 0.55 to 0.68 for PI, and from 0.43 to 0.66 for DP. Coefficient of variation values were
higher for VE and PI than DP on 13 May, 27 May and 24 Jun, but DP produced the highest CV on 10 Jun. Digital photography produced the lowest MSD values according to the Waller-Duncan k-ratio t-test on 3 of 4 assessment dates, whereas VE resulted in the highest MSD values on 3 of 4 assessment dates. In general, $r^2$, CV, and MSD values declined as SDS incidence declined due to turf recovery.

Analysis of variance detected significant differences ($p < 0.05$) among fungicides for all methods on all rating dates except for the 24 June rating date for the DP method. Mean square error values were lower for DP than VE and PI on all four assessment dates. The ratio of $\text{MSE}_{\text{VE}}$ or $\text{MSE}_{\text{PI}}$ to $\text{MSE}_{\text{DP}}$ indicates that VE resulted in between 1.51 to 11.05 times as much variation as DP, whereas PI produced 1.15 to 6.37 times more variable than DP. Analysis of variance was used to test for significant differences in MSE among assessment methods, using dates as replications. The $\ln\text{MSE}_{\text{DP}}$ was significantly lower than the $\ln\text{MSE}_{\text{VE}}$, but was not significantly lower than $\ln\text{MSE}_{\text{PI}}$.

Assessment method influenced the results of the Waller-Duncan k-ratio t-test, a common method for evaluation of treatment effects on disease incidence (Table 2). On 13 May, no significant differences among fungicides were detected using VE or PI, whereas DP detected differences among the untreated control and azoxystrobin, fenarimol, and propiconazole. Similar differences among rating methods were observed on 10 June. On 24 June, both VE and PI detected significant differences among treatments, whereas no differences were detected by DP. On this date, the turf was severely injured from scalping. DP could not accurately distinguish between scalping injury and SDS, while the experienced evaluators conducting VE and PI were able to discern between the two symptoms.
Linear regression of VE vs. DP over all rating dates produced the equation VE = 1.28DI – 0.018 (Figure 1). The y-intercept of -0.018 indicates that VE slightly underestimates when disease incidence is low, whereas the slope of 1.28 indicates that VE begins to overestimate as disease incidence increases. Regression of PI vs. DP resulted in the equation PI = 0.86DI + 0.365 (Figure 2). The PI method appears to slightly over-estimate when disease incidence is low, but underestimate when disease incidence is high, compared to DP.

Field #5 ‘Fungicides Study’

\( R^2 \) values ranged from 0.61 to 0.65 for VE, from 0.66 to 0.72 for PI, and from 0.63 to 0.73 for DP (Table 3). CV values were higher for VE and PI than DP on 13 May, 27 May and 24 Jun, but DP produced the highest CV on 10 Jun. DP produced lower MSD values than VE on 3 of 4 assessment dates, but was only lower than PI on 1 of 4 dates. In contrast to field #4, \( R^2 \), CV, and MSD values did not decline as SDS incidence declined due to turf recovery.

Analysis of variance detected significant differences (\( p < 0.05 \)) among fungicides for all methods on all rating dates except for the 24 June rating date for the VE method. Mean square error values were lower for DP than VE on all four assessment dates and lower than PI on 2 of 4 assessment dates. The ratio of MSE\(_{VE}\) or MSE\(_{PI}\) to MSE\(_{DP}\) indicates that VE resulted in between 2.13 to 8.53 times more variation than DP, whereas PI produced 0.35 to 1.22 times as much variation as DP. Analysis of variance was used to test for significant differences in MSE among assessment methods, using dates as
replications. The \( \ln\text{MSE}_{DP} \) was significantly lower than the \( \ln\text{MSE}_{VE} \), but was not significantly lower than \( \ln\text{MSE}_{PI} \).

On 13 May, no significant differences among fungicides were detected using DP or PI, whereas VE detected differences among SA 0.1 L m\(^{-2}\), SA 0.2 L m\(^{-2}\), and SA 0.4 L m\(^{-2}\) (Table 4). Similar differences among rating methods were observed on 27 May. As discussed for field #4, scalping injury on 24 June interfered with DP’s ability to detect SDS symptoms. Significant differences among treatments were detected by DP on this date but were not detected by VE or PI.

Linear regression of VE vs. DP over all rating dates produced the equation \( VE = 1.68DI - 1.996 \) (Figure 3). The y-intercept of -1.996 indicates that VE underestimates when disease incidence is low, whereas the slope of 1.68 indicates that VE begins to overestimate as disease incidence increases. Regression of PI vs. DP resulted in the equation \( PI = 0.87DI - 0.80 \) (Figure 4). The PI method appears to slightly over-estimate when disease incidence is low, but underestimate when disease incidence is high, compared to DP.

**DISCUSSION**

Research on SDS has been hampered by high experimental errors due to the irregular distribution of the disease in the field. Therefore, minimizing experimental error is essential to developing a better understanding of this host-pathogen relationship. The method used to assess disease incidence represents one potential source of experimental error. Visual estimation and PI are currently the standard methods for assessment of SDS incidence. Richardson et al. (2001) and Karcher et al. (2003) demonstrated that DP is a
more accurate and precise method than VE and PI for assessment of various turfgrass parameters.

Results of this study also indicated that DP is also more effective than VE for assessment of SDS incidence. Digital photography consistently produced higher $r^2$ values and lower CV, MSD, and MSE values than VE. Reductions in experimental error translated directly to differences in mean separations used to compare disease incidence in response to fungicide treatments. Digital photography was not consistently more accurate or precise than PI, as indicated by the lack of significant differences among MSE$_{DP}$ and MSE$_{PI}$. Point-intersect may represent an alternative to DP when this method is not economical or practical.

Some disadvantages of DP were noted during the execution of this research. On 24 June, the bermudagrass on Field #4 was severely scalped by mowing. The image analysis program was not able to accurately discern between SDS symptoms and scalping injury on this date. In this situation, VE and PI were more effective for detection of significant differences among treatments because the evaluators were trained in discerning SDS symptoms from scalping injury (Table 2).

Another disadvantage of DP in many applications is the effect of light quality on assessment results. Because of the distinct contrast between healthy turf and turf exhibiting SDS symptoms, light quality was not a significant factor in our situation. However, shadows over the plot from the monopod and operator could mask SDS symptoms in the images. Shadows could not be avoided between the hours of 1230 and 1430 in Raleigh, NC, therefore, images were not taken at this time. The sensitivity of camera equipment to precipitation is another problem, whereas VE and PI could be
Conducted in the rain if necessary. Finally, in order to completely assess a field trial containing 200 plots, DP required 4 hours of labor, whereas PI and VE could be completed in 3.3 and 1.75 hours, respectively.

Methods for assessment of SDS incidence differ in their accuracy and precision. Selection of assessment methods can influence the researcher’s ability to detect significant differences among treatments in field research. Overall, DP appears to be the most effective and efficient method for assessment of SDS incidence in bermudagrass. If DP is not an option, then PI is the next most acceptable method of assessment. VE is the least accurate assessment method and should not be used to assess SDS incidence. Digital photography could have additional applications in turfgrass pathology where there is a distinct contrast between healthy and diseases turf, such as with dollar spot caused by *Sclerotinia homoeocarpa*. The accuracy of DP for assessment of other diseases, such as brown patch (*Rhizoctonia solani*), where there is no distinct contrast between healthy and diseases turf, warrants further investigation.

**Acknowledgements**

This research was supported by the Center for Turfgrass Environmental Research and Education at North Carolina State University. The authors thank the Raleigh Parks and Recreation Department for their cooperation throughout this study. Valuable technical assistance was provided by David Lee, Charles Campbell, Brandon Cawthorne, Mac Malloy, and Patrick Gregg.
LITERATURE CITED


Figure 1. Linear regression of digital photography rating method vs. visual estimation method on Field 4 at Walnut Creek Softball Complex, Raleigh, NC.

\[ VE = 1.28DP - 0.018 \]
Figure 2. Linear regression of digital photography rating method vs. point-intersect method on Field 4 at Walnut Creek Softball Complex, Raleigh, NC.
Figure 3. Linear regression of digital photography rating method vs. visual estimation method on Field 5 at Walnut Creek Softball Complex, Raleigh, NC.
Figure 4. Linear regression of digital photography rating method vs. point-intersect method on Field 5 at Walnut Creek Softball Complex, Raleigh, NC.
Table 1. Statistical comparisons of three rating methods for SDS of bermudagrass: visual estimation (VE), point-intersect (PI), and digital photography (DP) across four dates in 2003.

<table>
<thead>
<tr>
<th></th>
<th>VE</th>
<th>PI</th>
<th>DP</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-May</td>
<td>0.70 0.69 0.64 0.53</td>
<td>0.62 0.68 0.65 0.55</td>
<td>0.66 0.64 0.54 0.43</td>
</tr>
<tr>
<td>27-May</td>
<td>79.62 100.52 158.90</td>
<td>94.29 83.96 90.24 107.94</td>
<td>64.66 63.67 125.08 43.72</td>
</tr>
<tr>
<td>10-Jun</td>
<td>14.57 9.69 4.42 0.94</td>
<td>7.86 8.72 3.30 0.79</td>
<td>4.57 5.93 0.81 1.09</td>
</tr>
<tr>
<td>24-Jun</td>
<td>p-value &lt; 0.0001 &lt; 0.0001 &lt; 0.0001</td>
<td>0.0002 &lt; 0.0001 &lt; 0.0001</td>
<td>&lt; 0.0001 &lt; 0.0001 &lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>MSE 77.76 38.15 14.25 1.62</td>
<td>20.22 27.36 8.22 1.23</td>
<td>19.01 17.28 1.29 1.07</td>
</tr>
<tr>
<td></td>
<td>(σ²VE or σ²PI)/σ²DP</td>
<td>(σ²VE or σ²PI)/σ²DP</td>
<td>(σ²VE or σ²PI)/σ²DP</td>
</tr>
<tr>
<td></td>
<td>lnMSE (lnσ²)† 2.73 a 2.25 ab</td>
<td>1.53 b</td>
<td>2.25 ab</td>
</tr>
</tbody>
</table>

† Variance for each method on individual rating datings after removing plot effects.
‡ Comparison of variances among VE or PI to DP by dividing MSEVE or MSEPI by MSED on respective rating dates.
§ Natural log (ln) of MSE for each respective rating method and then subjected to ANOVA, using assessment dates as replicates.
Means within columns followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).
Table 2. Differences in means separation among three rating methods (visual estimation (VE), point-intersect (PI), and digital photography (DP)) for fungicide efficacy on SDS of bermudagrass across four dates in 2003.

<table>
<thead>
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<th></th>
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<th>PI T</th>
<th>DP T</th>
</tr>
</thead>
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<tr>
<td>Azoxyostrobin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13-May</td>
<td>A</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>27-May</td>
<td>A</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>10-Jun</td>
<td>A</td>
<td>A</td>
<td>AB</td>
</tr>
<tr>
<td>24-Jun</td>
<td>A</td>
<td>A</td>
<td>AB</td>
</tr>
<tr>
<td>Fenarimol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13-May</td>
<td>A</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>27-May</td>
<td>A</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>10-Jun</td>
<td>A</td>
<td>A</td>
<td>BC</td>
</tr>
<tr>
<td>24-Jun</td>
<td>A</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Myclobutanil</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>AB</td>
</tr>
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</tr>
<tr>
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<td>A</td>
<td>A</td>
<td>AB</td>
</tr>
<tr>
<td>24-Jun</td>
<td>A</td>
<td>A</td>
<td>AB</td>
</tr>
<tr>
<td>Propiconazole</td>
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<td></td>
<td></td>
</tr>
<tr>
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</tr>
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</tr>
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</tr>
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</tr>
<tr>
<td>Untreated Control</td>
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<tr>
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<td>A</td>
<td>A</td>
</tr>
<tr>
<td>27-May</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>10-Jun</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>24-Jun</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
</tbody>
</table>

† Values are the means of 8 replicates. Means within columns followed by the same letter are not significantly different according to the Waller-Duncan k-ratio t-test (k=100).
<table>
<thead>
<tr>
<th>Date</th>
<th>VE</th>
<th>PI</th>
<th>DP</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-May</td>
<td>0.65</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td>27-May</td>
<td>0.64</td>
<td>0.68</td>
<td>0.73</td>
</tr>
<tr>
<td>10-Jun</td>
<td>0.62</td>
<td>0.72</td>
<td>0.67</td>
</tr>
<tr>
<td>24-Jun</td>
<td>0.61</td>
<td>0.66</td>
<td>0.63</td>
</tr>
<tr>
<td>CV</td>
<td>82.83</td>
<td>90.71</td>
<td>46.03</td>
</tr>
<tr>
<td>MSD</td>
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<td>4.46</td>
<td>4.44</td>
</tr>
<tr>
<td>p-value</td>
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<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>MSE(σ²) †</td>
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<td>27.25</td>
<td>14.16</td>
</tr>
<tr>
<td>lnMSE(σ²) §</td>
<td>2.88 a</td>
<td>3.40 b</td>
<td>1.63 b</td>
</tr>
</tbody>
</table>

† Variance for each method on individual rating datings after removing plot effects.
‡ Comparison of variances among VE or PI to DP by dividing MSEVe or MSEPI by MSEDP on respective rating dates.
§ Natural log (ln) of MSE for each respective rating method and then subjected to ANOVA, using assessment dates as replicates.
Means within column followed by the same letter are not significantly different according to Waller-Duncan k-ratio t-test (k=100).
Table 4. Differences in mean separation among three rating methods (visual estimation (VE), point-intersect (PI), and digital photography (DP)) for application method efficacy on SDS of bermudagrass across four dates in 2003.

<table>
<thead>
<tr>
<th></th>
<th>VE†</th>
<th>PI†</th>
<th>DP†</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-May</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>27-May</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>10-Jun</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>24-Jun</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>13-May</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27-May</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>10-Jun</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>24-Jun</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>SA 0.1 L m⁻²‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Cushman Envirojet‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>AB</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>AB</td>
<td>AB</td>
<td>A</td>
<td>A</td>
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</tbody>
</table>

† Values are the means of 5 replicates. Means within columns followed by the same letter are not significantly different according to the Waller-Duncan k-ratio t-test (k=100).
‡ Fungicides were applied by one of five different application methods: surface applied (SA) in a water-carrier volume of either 0.1 L m⁻², 0.2 L m⁻², 0.4 L m⁻², 0.1 L m⁻² followed by 6 mm irrigation (WI), or high-pressure soil-injection (Cushman Envirojet).
CHAPTER FOUR

DISTRIBUTION OF OPHIOSPHAERELLA SPECIES CAUSING SPRING DEAD SPOT IN NORTH CAROLINA
INTRODUCTION

Spring dead spot (SDS) is one of the most severe diseases of bermudagrass (*Cynodon dactylon* (L.) Pers. and *C. dactylon* x *C. transvaalensis* Burtt-Davy) in Australia, New Zealand, and in the United States where bermudagrass goes into winter dormancy. Spring dead spot is caused by the ectotrophic, root-infecting fungi *Ophiosphaerella korrae* Walker and Smith (synonym *Leptosphaeria korrae* Walker and Smith), *O. herpotricha* (Fr.), and *O. narmari* Walker and Smith (synonym *L. narmari* Walker and Smith) (Crahay et al., 1988; Endo et al., 1985; Smith, 1971; Tisserat et al., 1989; and Walker & Smith, 1972; Wetzel et al., 1999 a). *Ophiosphaerella herpotricha* is the most common causal agent of SDS in Kansas, Oklahoma, and Texas (Tisserat et al. 1989), whereas *O. korrae* is the predominant pathogen in Maryland (Crahay et al., 1988). The causal organism for SDS in North Carolina has been reported to be *Gaeumannomyces graminis* (McCarty & Lucas, 1989).

Spring dead spot usually does not appear until a stand of bermudagrass is more than two years old (Couch, 1995). Symptoms of SDS appear in the spring as bermudagrass resumes growth from winter dormancy. Typical field symptoms are circular patches or rings of brown to straw-colored turf ranging from 15 cm to several meters in diameter. Plants within patches remain dormant and eventually die, collapsing to the ground. Individual patches reappear annually, gradually expand in size, and often coalesce to form larger areas of afflicted turf. The patches may not be easily detected where bermudagrass has been over seeded or in stands with high populations of winter weeds (Couch, 1995). On individual plants, extensive necrosis of stolons, rhizomes, and roots is evident at the time of symptom expression. The majority of infection and
colonization is thought to occur in the fall, which may predispose the bermudagrass to winter injury (Lucas, 1980; Lucas & Gilbert, 1979).

Diagnosis of SDS is typically performed based on field symptoms and observation of necrotic roots, stolons, and or rhizomes. In order to obtain a definitive diagnosis, however one must confirm the presence of a particular pathogen. *Ophiosphaerella* spp. are members of the ectotrophic root-infecting fungi (ERI). Ectotrophic root infecting fungi colonize roots before penetrating the vascular tissue of the host. Typically, ERI fungi incite necrosis of the roots, stolons and rhizomes of turfgrass host plants. These pathogens cause patch symptoms on many turfgrasses and include species from the genus *Gaeumannomyces, Ophiosphaerella, Leptosphaeria, Magnaporthe*, and *Phialophora* (Wetzel et al., 1996). These fungi produce few distinctive morphological features that can be used for identification. Typically, all of these organisms produce dark brown to black septate hyphae that grow along roots or stolons. Some species produce infection structures, called hyphopodia, which may be used for identification. The only way to positively identify many ERI fungi is to induce production of the sexual stage *in vitro*. However, this is very time consuming and often unsuccessful. Another method for identification of ERI fungi is culture morphology. Wetzel et al. (1996) showed that *O. korrae* colonies exhibited raised or dome-shaped mycelium and *O. herpotricha* exhibited brownish-black colored exudates in the middle of 2-week-old cultures. Identification based on colony morphological features alone may work, but it is difficult to definitively identify these organisms on those features alone.

More recently, methods for identification of *Ophiosphaerella* species using molecular techniques have included RAPD-PCR. Wetzel et al. (1996) used this
technique to identify common ERI fungi such as *Gaeumannomyces* spp., *Magnaporthe* sp., *Ophiosphaerella* spp., and *Phialophora* sp. However, RAPD-PCR does not have the specificity of modern PCR techniques and therefore is considered an out-of-date technique.

Wetzel et al. (1999 a) demonstrated a technique for identification of *Ophiosphaerella* spp. using PCR primers ITS4 and ITS5, which amplify the ITS1-5.8S-ITS2 region of the rDNA. Tisserat et al. (1994) found that by using universal primers ITS4 and ITS5, *O. herpotricha* isolates always resulted in a 590-bp DNA fragment and *O. korrae* isolates yield either a 590 or 1,019-bp DNA fragment. Based on sequences of the ITS1-5.8S-ITS2 regions, specific PCR primers were developed for *Ophiosphaerella korrae* and *O. herpotricha*. *O. herpotricha* primers, OH1 and OH2, amplified a 454-bp fragment from *O. herpotricha* DNA and not from 29 other fungal and bacterial species tested, including *O. korrae*. Primers OK1 and OK2 are specific for *O. korrae*, and amplified a 454-bp fragment was amplified from *O. korrae* DNA and not from 29 other fungal and bacterial species tested, including *O. herpotricha*.

In a study conducted by Wetzel et al. (1999 b) to evaluate the geographic distribution of *Ophiosphaerella* spp. in the Mid-West, *O. herpotricha* (445 of 531) was the most abundant organism isolated from samples taken from three sites. *O. korrae* (47 of 531) and *O. narmari* (21 of 531) were also detected at much lower levels.

Correct etiology of any plant disease is critical in understanding the epidemiology of the disease and the nature of the interaction between the host and pathogen. The etiology of SDS in North Carolina is not fully known and therefore needs to be investigated. The objective of this research was to determine the distribution of
*Ophiosphaerella* species causing SDS in North Carolina. This is important to determine because *O. herpotricha* has been shown to be more aggressive and potentially more difficult to control when compared to the other *Ophiosphaerella* spp. (Tisserat et al., 2004).

**MATERIALS & METHODS**

One-hundred ninety-six isolates of a tentative pathogen were isolated from plants with symptoms of spring dead spot (SDS) in the spring of 2003 and twenty-five isolates were collected in the spring of 2004 from five regions in North Carolina following complete green-up of bermudagrass (Figure 1). At each site, areas of moderate to heavy SDS infestation were scouted. In 2003, three golf balls were thrown collectively into the air within the area of infestation and the patch closest to each individual ball was sampled on the outer edge of the symptomatic patch, one half healthy and one half symptomatic, with a Par-Aide (Lino Lakes, MN 55038) golf course cup cutter (11 cm diameter) to a depth of just below the bottom of the root zone. In 2004, an arbitrary sampling pattern was used based on patch size. Patch diameters were measured in two directions and averaged, then turf cores were taken from the outer edge of the symptomatic turf patch, with one half healthy and one half symptomatic.

Samples were taken to the laboratory within one day for diagnosis and isolation of the suspected causal agent. Samples were split into quarters and all soil was washed from the roots using de-ionized water (dH₂O). Stolons and roots were analyzed for the morphological characteristics of the suspected causal agent. Five to 10 mm tissue sections were taken from all regions that exhibited necrotic lesions or ectotrophic hyphae.
Tissue sections were washed in a cheese-cloth covered beaker under constant water flow from a de-ionized water tap for 10 minutes. Tissue sections were then submersed in 10% Clorox for 5 minutes, rinsed with sterile dH$_2$O, and blotted dry with paper towels. Tissue sections were then plated on ¼ strength potato dextrose agar amended with streptomycin sulfate (100 mg/L) and chloramphenicol (100 mg/L) and allowed to grow for 5-7 days at room temperature (23 to 25°C). Colonies resembling *Ophiosphaerella* were then transferred to potato dextrose agar (PDA ++++) amended with streptomycin sulfate (50 mg/L), chloramphenicol (50 mg/L), and tetracycline (50 mg/L) and allowed to grow at room temperature for 14 days. Pure cultures of the suspected casual agent, based on colony morphology alone, were then transferred to PDA +++ that contained pieces of sterilized filter paper and allowed to grow at room temperature until all pieces of filter paper were covered by hyphal growth, usually around 14 days. The infested filter paper pieces were then removed, placed into a small coin envelope, dried overnight in a laminar flow hood overnight and then placed into a -80 °C freezer for storage.

For fungal DNA isolation, samples were plated onto PDA+++ and allowed to grow for 4-5 days. Samples were hyphal tipped into 4 sections (< 1 mm) and grown for 7 to 10 days in 2 mL of potato dextrose broth on a bench top shaker (~200 rpm). The mycelium suspension was then transferred to 1.5 ml micro-centrifuge tubes, and harvested after centrifugation for 5 min. at 14,000 rpm. Genomic DNA was extracted with the Easy-DNA Kit (Invitrogen Corp., Carlsbad, CA).

Species-specific PCR primers for *O. herpotricha* and *O. korrae* are described by Tisserat et al (1994). The sequences for *O. herpotricha* primers are as follows in the 5’ to 3’ direction: OH1, CCAAGTGTAGAACAAACTACGC and OH2, AAAAGGCTTA.
TTGGGTGCCTAT. *Ophiophaerella korrae* primers are as follows in the 5’ to 3’ direction: OK1, CCAAGTGCAAGCACAACACTGATG and OK2, AAGAGGCTTAATAAGGTGCACAC. Species-specific primers for *O. narmari* are described by Wetzel et al (1999a). The sequences for *O. narmari* primers are as follows in the 5’ to 3’ direction: ON1, CCAAGYGTTAGAACAACTAT and ON2, GGTCGACTGATAAAAGGG. Wetzel et al (1999a) describes the ‘Y’ in ON1 as a degenerate 50% C and 50% T base composition due to variability of the nucleotide among *O. narmari* isolates at that position. The PCR conditions were the same for *O. herpotricha* and *O. korrae* and are as follows, Step 1, 94 °C for 1 min, Step 2, 94 °C for 30 sec, Step 3, 65 °C for 1 min, Step 4, 72 °C for 1 min, Step 5, 32 times repeat to Step 2, Step 6, 72 °C for 2 min, and Step 8, hold at 4 °C. The composition of the PCR reaction is as follows; 10X PCR buffer = 0.1 volumes, 50 mM MgCl₂ = 1.5 mM, 5 U/µl taq-polymerase = 0.625 U, 2.5 mM dNTPs = 200 uM, 10 µM ITS1 primer = 200 nM, 10 µM ITS2 primer = 200 nM, 50 ng/µl template DNA = 50 ng, and dH₂O = 25 µl. 1 µl template DNA was added to 24 µL of master mix and then placed in the thermocycler. PCR conditions for *O. narmari* are as follows; Step 1, 94 °C for 2 min, Step 2, 94 °C for 30 sec, Step 3, 61 °C for 30 sec, Step 4, 72 °C for 30 sec, Step 5, 15 times repeat to Step 2, Step 6, 94 °C for 30 sec, Step 7, 60 °C for 25 sec, Step 8, 72 °C for 1 min, Step 9, 14 times repeat to Step 6, Step 10, 72 °C for 6 min, and Step 11, hold at 4 °C.

Following PCR, amplified samples were confirmed using electrophoresis at 110 V for 30 min in a 1% agarose gel. DNA products were viewed with UV light and photographed for record.
RESULTS

In 2003, of the 196 isolates collected it was confirmed that 160 isolates were *O. korrae*, 22 were *O. herpotricha*, and 0 were *O. narmari*. The remaining 14 isolates remain to be identified, but were not identified using these primer sets. Across all regions sampled, *O. korrae* was the dominant species (Table 1). All sites sampled tended to provide only one species, except for one site each in the Raleigh and Wilmington regions. In the Charlotte region at Renaissance GC, all isolates were *O. herpotricha*. In the Raleigh region at the NC State University Turfgrass Field Lab, *O. herpotricha* was the dominant pathogen, but from a single turf core from different roots, both *O. herpotricha* and *O. korrae* were discovered. In the Wilmington region at Lockwood Folly, *O. korrae* was the dominant pathogen and *O. herpotricha* was discovered at this site, but not from the same turf core.

In 2004, three particular sites were sampled again due to the results from 2003 (Table 2). Sample locations included Landfall due to variance in patch color, and Renaissance and Lockwood Folly due to detection of *O. herpotricha* in 2003. Of the 25 isolates collected from these three sites, it was confirmed that 25 isolates were *O. korrae*. Patch diameters were noted to potentially detect differences in patch size related to species. Patch diameters ranged from 15.24 – 68.58 cm (Landfall, µ = 40.64 cm), 20.32 – 134.62 (Renaissance, µ = 78.90 cm), and 15.24 – 119.38 cm (Lockwood Folly, µ = 67.31).
DISCUSSION

In 2003, it was confirmed that the majority of isolates collected across all five regions of North Carolina are *O. korrae*. Some isolates were identified as *O. herpotricha*. None of the isolates in this study were *O. narmari*. Based on this result, *O. korrae* is the predominant species of *Ophiosphaerella* in North Carolina that causes SDS of bermudagrass.

Of interest in the 2003 collection are those sites in which we identified both *O. korrae* and *O. herpotricha*. In most cases, the two species were found in separate turf cores. However, at the NCSU Field Laboratory, both *O. korrae* and *O. herpotricha* were recovered from the same turf core. This is also the site with the highest incidence of *O. herpotricha* occurring in the survey. Since this is a research farm site, there may have been SDS work conducted on these greens in the past and could have potentially been inoculated with unknown strains of SDS. Therefore, it was decided to ignore the data from this site with regards to the overall study. Of interest though, is the fact that *O. korrae* and *O. herpotricha* were identified from different roots within the same turf core. Wetzel et al. (1999 b) found similar results on several occasions in their study and also found multiple species within a given sample location. Further study into this matter would have to be conducted, but it may be possible for multiple species of SDS to co-exist within a given area of infection.

In 2003, 14 isolates could not be identified with the primer sets used. All of these isolates appeared to be the causal organism based on colony morphology. This could indicate that the primer sets may miss some SDS isolates. Further research is needed to
characterize these isolates and determine if modifications to the specific PCR primers are needed.

In 2004, all isolates collected were identified as *O. korrae*. This is of interest because sampling of these areas was conducted as a follow up to the 2003 data set. Wetzel et al. (1999 b) sampled the same site in multiple years and found differences in population composition among sample years; it is possible this may vary from year to year due to weather conditions. In 2003 at the Renaissance site, all isolates were *O. herpotricha*. In 2004 at the Renaissance site, all isolates were *O. korrae*. In both years sampling was conducted randomly or arbitrarily, therefore it would be a matter of chance for which species one could isolate. Since SDS patches are highly abundant and widespread at this site, more intense research at this site must be conducted before conclusions can be drawn. Obviously this is an issue that requires further research in the coming years.

In 2004, at the Landfall site, the interest in sampling was due to distinct differences in patch morphology. This site contained patches of various sizes and colors in the spring of 2003. Since all isolates were identified as *O. korrae*, no relationship can be determined with regards to patch size or color in this study.

In conclusion, the PCR based assay for SDS described by Wetzel et al (1999 a) and Tisserat et al (1994) successfully identified the causal organism of SDS is in North Carolina as *O. korrae*. However, the protocols for *O. herpotricha* and *O. korrae* were developed primarily for isolates not collected in North Carolina. Several isolates collected in this study remain unidentified and require further investigation to determine
if they are species of *Ophiosphaerella* that are not identified with the previously developed PCR primers.

These results provide vital information as future research proceeds on SDS of bermudagrass in North Carolina. Knowing the etiology of SDS of bermudagrass will assist with future epidemiology and/or control studies with this plant disease in North Carolina.

**Acknowledgements**

The authors would like to thank Matt Martin, Jim Monroe, and Eric Honeycutt with the North Carolina Cooperative Extension Service, Trey Warnock with Bayer Environmental Science, and all of the superintendents at the sites which were sampled for their efforts in collecting samples.

**LITERATURE CITED**


Table 1. SDS populations from bermudagrass cultivars in North Carolina sampled in 2003 as determined by a polymerase chain reaction based assay.

<table>
<thead>
<tr>
<th>Location</th>
<th>Region</th>
<th>Cultivar</th>
<th>O. korrae</th>
<th>O. herpotricha</th>
<th>O. narmari</th>
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<td>Biltmore Forest</td>
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<td>Tifway</td>
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| Total             | 160     | 22       | 0         | 14               |

*Regions according to corresponding Figure 1.*
Table 2. SDS populations from bermudagrass cultivars in North Carolina sampled in 2004 as determined by a polymerase chain reaction based assay.

<table>
<thead>
<tr>
<th>Location</th>
<th>Region</th>
<th>Cultivar</th>
<th><em>O. korrae</em></th>
<th><em>O. herpotricha</em></th>
<th><em>O. narmari</em></th>
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<td><strong>25</strong></td>
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*Regions according to corresponding Figure 1.*
Figure 1. Map of North Carolina showing regions that were sampled in 2003 and 2004. Region 1 = Asheville area, Region 2 = Charlotte area, Region 3 = Pinehurst area, Region 4 = Raleigh area, and Region 5 = Wilmington area.
CHAPTER FIVE

SUMMARY AND CONCLUSIONS
Spring dead spot is a severe patch disease of bermudagrass (*Cynodon dactylon* (L.) Pers. and *C. dactylon* x *C. transvaalensis* Burtt-Davy), caused by *Ophiosphaerella herpotricha, O. korrae, O. narmari*. The disease was first observed in Oklahoma in 1936 (Smith et al., 1989), but was not officially named spring dead spot until 1960 by Wadsworth & Young (1960). This disease is known to occur in New South Wales, Australia and regions of the United States where bermudagrass goes into winter dormancy (Couch, 1995). It has been recorded in Alabama, Arkansas, California, North Carolina, South Carolina, Georgia, Kansas, Maryland, Mississippi, Missouri, Nebraska, Tennessee, Texas, and Virginia (Couch, 1995). *Ophiosphaerella korrae* is also a pathogen of Kentucky bluegrass (*Poa pratensis* L.), bentgrass (*Agrostis* spp.), creeping red fescue (*Festuca rubra* L.), annual bluegrass (*Poa annua* L.), and rough bluegrass (*Poa trivialis* L.), causing a disease commonly known as necrotic ring spot on these hosts (Smiley & Fowler, 1984; Worf et al., 1986; Smiley et al., 1992).

Bermudagrass is used in North Carolina from the Piedmont to the Coastal Plain in various situations where turf can be grown. It is the primary grass used for high maintenance landscapes and playing surfaces for golf, baseball, football and soccer. Since North Carolina is situated in the transition zone (Turgeon, 1991), growers are faced with the daunting task of growing this warm season grass in a region that has a winter season. In addition to the potential for injury during the winter from cold, freezing, or desiccation, bermudagrass is highly susceptible to attack from fungal diseases, including spring dead spot (SDS), during the fall and spring.

As with any plant disease, it is imperative that as much as possible is understood about the causal organism, its biology, and its interactions with the host plant. There are
many key factors that are unknown about SDS in North Carolina. For example, the identity of the causal agent in North Carolina was unknown until this research project. Etiology of a disease is critical to understanding epidemiology, therefore making control programs difficult to predict or recommend. Current cultural recommendations improve the health and growing conditions of bermudagrass and have been shown to reduce predisposition to SDS, but do not provide adequate results alone. Chemical control recommendations are limited and vague. When chemicals are used, results are highly variable. Therefore, it is easy to understand why SDS control is difficult and frustrating for turfgrass managers. This project was based on the need to answer such critical questions about SDS of bermudagrass. The objectives of this research were to develop effective and specific recommendations for control of SDS, to compare methods for assessment of SDS incidence, and to determine the etiology of SDS in North Carolina.

Spring dead spot can be managed with an integrated approach, implemented over a period of several years. Improving soil conditions, proper nitrogen fertilization, fall potassium applications, and reduction of soil pH are effective options for reducing SDS severity. Preventative fungicide applications are an option in high value areas or where cultural practices alone do not provide adequate control. Of the fungicides that were evaluated in this project, fenarimol and propiconazole appear to be the most effective providing from 44 to 89% and 42 to 54% control respectively.

No significant differences were detected among fungicide application methods in this study, but applications in higher volumes of water or when watered-in tended to provide better control when compared to the standard application of 0.1 L m\(^{-2}\) or use of the Envirojet. Since \textit{O. korrae} attacks the below-ground tissues of bermudagrass,
application methods that deliver the fungicide closer to the causal organism are expected
to be more effective. However the Envirojet did not significantly improve SDS control
and does not support this theory. The Envirojet delivers product on 5 cm spacings,
therefore it is possible that this is too far apart and the chemical is not applied uniformly
enough.

The precise timing of fungicide applications does not appear to be critical, as long
as they are made before soil temperatures fall below 15 °C. This would correlate with the
optimal temperatures for pathogen growth, which range from 14 to 30 °C. Also at this
temperature, bermudagrass is still active, therefore allowing the plant to uptake the
fungicides. Also, control of SDS tended to be better when multiple applications were
made starting in August or September.

Based on the results of this objective, control of SDS with fungicides is possible.
Unlike some diseases, SDS typically can not be totally eradicated in one year. The use of
the proper fungicide, application method, and application timing along with proper
cultural practices over several years will ensure a satisfactory level of SDS control.

In 2003 and 2004, it was confirmed that the majority of isolates collected across
all five regions of North Carolina are *Ophiosphaerella korrae*. Results also identified
some isolates as *O. herpotricha*. None of the isolates in this study were *O. narmari*.
Based on this result, *O. korrae* is the predominant species of *Ophiosphaerella* in North
Carolina that causes SDS of bermudagrass. This data is vital information for future
research on SDS of bermudagrass in North Carolina. Now that the causal organism has
been confirmed as *O. korrae*, future endeavors into this area of research should be greatly
aided with this knowledge. Knowing the etiology of SDS of bermudagrass will assist with future epidemiology and/or control studies with this plant disease in North Carolina.

Research of SDS has been hampered by high experimental errors due to the irregular distribution of the disease in the field. Therefore, minimizing experimental error is essential to developing a better understanding of this host-pathogen relationship. The method used to assess disease incidence represents one potential source of experimental error. Results of this study indicate that digital photography (DP) is more effective than visual estimation (VE) for assessment of SDS incidence. Digital photography consistently produced higher $r^2$ values and lower CV, MSD, and MSE values than VE. Reductions in experimental error translated directly into differences in mean separations used to compare disease incidence in response to fungicide treatments. Digital photography was not consistently more accurate or precise than a point-intersect (PI) method, as indicated by the lack of significant differences among MSE$_{DP}$ and MSE$_{PI}$. PI may represent an alternative to DP when this method is not economical or practical.

Methods for assessment of SDS incidence differ in their accuracy and precision. Selection of assessment methods can influence the researcher’s ability to detect significant differences among treatments in field research. Overall, DP appears to be the most effective and efficient method for assessment of SDS incidence in bermudagrass. If DP is not an option, then PI is an alternative. Visual estimation is the least accurate assessment method and should not be used to assess SDS incidence.

In conclusion, the efforts of this program were able to answer all of the objectives initially stated. This research project will assist current and future researchers and managers of bermudagrass turf. Future research on SDS should investigate economically
sound control options as the fungicide market changes and as rules and regulation change concerning pesticide applications occur in the coming years. Future research also should evaluate SDS incidence assessment methods more in-depth to potentially refine the best methods available now to provide accurate and precise data results. Finally, future research should study spatial and temporal aspects of SDS for clarification and understanding of SDS epidemiology.

LITERATURE CITED


