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**TOWARDS REDUCING FUNGICIDE USE IN THE CONTROL OF
DOLLAR SPOT (SCLEROTINIA HOMOEOCARPA F.T. BENNETT)
DISEASE ON CREEPING BENTGRASS (AGROSTIS STOLONIFERA
L.)**

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ABSTRACT OF THESIS

TOWARDS REDUCING FUNGICIDE USE IN THE CONTROL OF DOLLAR SPOT (*SCLEROTINIA HOMOEOCARPA* F.T. BENNETT) DISEASE ON CREEPING BENTGRASS (*AGROSTIS STOLONIFERA* L.)

Creeping bentgrass (*Agrostis stolonifera* L.) is commonly used on golf course greens and fairways in cool-humid regions but is plagued by numerous fungal diseases, one of which is dollar spot disease (*Sclerotinia homoeocarpa* F. T. Bennett). Dollar spot occurs frequently throughout the growing season requiring biweekly fungicide applications for complete control. The objective of this study was to investigate methods of reducing the number of fungicide applications needed to maintain dollar spot at acceptable levels through dew removal and potential mechanisms of resistance in bentgrass. In the first study, a combination of mowing three times a week and dragging by hose the remaining four days to remove dew was used in an attempt to reduce disease severity. The main effect of this combination treatment was not significant ($p>0.05$) and did not reduce the number of fungicide applications compared to normal mowing three times a week. However, dollar spot was managed curatively with 20-80% fewer applications compared to a normal preventative fungicide program. In the second experiment, two experimental germplasms with varying disease resistance were tested for the possible production of antifungal compounds known as phytoanticipins. Preliminary results indicate the resistant line may contain compounds not present in the susceptible line.

KEYWORDS: Creeping Bentgrass, Dollar Spot, Dew Removal, Disease Resistance, Phytoanticipins

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4 May 2009

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BENTGRASS (*AGROSTIS STOLONIFERA* L.)

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THESIS

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture
at the University of Kentucky

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2009

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Chapter One

Literature Review

Introduction

Creeping bentgrass (*Agrostis stolonifera* L.) is a cool-season turfgrass that is commonly found throughout cool-humid regions. It is used on almost all golf course greens and many fairways due to its ability to withstand low heights of cut, excellent playability, and because of its good color and texture. While bentgrass has these desirable characteristics, it is also a very high-maintenance grass. It can be difficult to maintain due to its potential thatch problems, need for frequent irrigation, and disease problems. Among the many diseases that can affect creeping bentgrass, one of the largest problems is dollar spot disease (caused by *Sclerotinia homoeocarpa* F. T. Bennett).

Dollar spot is a fungal disease that affects several turfgrasses, especially creeping bentgrass. In short-mowed turf, symptoms of dollar spot include small dollar-size patches that are chlorotic or straw-colored and chlorotic leaf lesions which eventually become bleached or tan colored and have reddish brown margins (Smiley et al., 2005). If left untreated, the patches can increase in number and begin coalescing forming large diseased areas. *S. homoeocarpa* forms mycelium which allows the pathogen to spread by aerial mycelial growth to neighboring plants during periods of warm days and cool nights. It can also be spread across larger areas by humans and machinery. Once the mycelium contacts another plant, it can enter through the stomates or through tissue cut from mowing or other injuries (Smiley et al., 2005). The mycelium is most visible during periods of heavy dew and can appear similar to spider webs on the surface of the turf.

Unless the disease becomes very severe, tillers are usually not killed and most of the damage is aesthetic, but this still creates a major problem for golf courses.

Chemical Control of Dollar Spot Disease

In very high-maintenance turf systems, a pest that may not kill the grass but negatively impacts its quality can often be as much of a concern as something that does kill the grass. While it may be impossible to create a 'perfect' turf, disruptions in the color, texture, density, and overall uniformity of the turf must be minimized as much as possible. It is because of this that dollar spot is such a concern for many golf courses and the reason why many chemicals are utilized to combat the fungus.

In many cases, the most common way to control a pest on a golf course is the application of chemicals. Golf courses deal with numerous pests including diseases, insects and weeds. Most of these pests can be controlled by a certain type of chemical or chemical combination, and dollar spot is no exception. Some of the current chemicals used to prevent and control dollar spot disease are fosetyl-AI, chlorothalonil, and iprodione, among others (Vincelli and Powell, 2007). While these fungicides have been found to be effective in controlling dollar spot, it may be possible that other chemicals or combinations of chemicals currently in use could provide equal or superior control. Several studies have examined if other chemicals used on golf courses such as plant growth regulators could provide dollar spot prevention and control.

A study conducted by McDonald et al. (2006) examined the effects of chlorothalonil, paclobutrazol (a plant growth regulator), and a wetting agent on dollar spot severity. The authors found that when the three chemicals were tested separately, chlorothalonil provided the best dollar spot control with paclobutrazol providing less

control and the wetting agent providing very little control. When chlorothalonil was combined with either paclobutrazol or the wetting agent or both, dollar spot control was usually better than chlorothalonil alone. This led the authors to conclude that paclobutrazol and the wetting agent could also be used to combat dollar spot. Fidanza et al. (2006) conducted a similar study by examining tank mixes of fungicides with plant growth regulators such as paclobutrazol and trinexapac-ethyl. They also found that when fungicides such as chlorothalonil were combined with plant growth regulators that dollar spot control was greater than with fungicides alone, but to a larger extent than McDonald et al. (2006) reported. Burpee and Latin (2008) examined possible synergisms among fungicides such as propiconazole, triadimefon, iprodione, vinclozolin, and chlorothalonil against dollar spot but could not find definitive proof of synergism among the chemicals tested.

In addition to discovering chemicals that provide enhanced pest control over current products, research will sometimes reveal chemicals that can increase damage from diseases such as dollar spot. As previously mentioned, numerous chemicals are used on golf courses and sometimes these chemicals can have the effect of increasing other pest problems. An example of this is the commonly used fungicide azoxystrobin which research has shown will allow dollar spot disease to increase, most likely by eliminating many other competing fungi in the soil (Hsiang and Cook, 2005; Vincelli and Powell, 2007).

Knowing which chemicals will work best against a pest, or perhaps even increase damage is very important. It is also beneficial for turf managers to understand how well their turf will recover from pest damage after chemical treatments have been applied. A

study conducted by Vincelli et al. (1997) examined how well 15 different cultivars of creeping bentgrass recovered from dollar spot outbreaks when left untreated or when treated with a fungicide. The study was conducted for three years and different results were observed each year. One year a cultivar might recover fairly well without a fungicide application following an outbreak of dollar spot and the next year it would not recover nearly as well. The authors believed this difference to be due to differences in temperatures and other environmental conditions among the years. One thing they were able to note though, is that little correlation was found between genetic resistance to dollar spot and recovery from it. While it is hard to draw any definitive conclusions of which cultivar is the absolute best in recovering from dollar spot since differences in management practices and environmental conditions will affect this, the authors concluded that the data they obtained may provide helpful guidelines for turf managers interested in how fast and how well their creeping bentgrass may recover from dollar spot infections.

Reducing the Use of Fungicides for Dollar Spot Control

While it is well known that various chemicals can be used to maintain dollar spot at acceptable levels, many studies have been conducted to attempt to find alternative ways to control the disease, or at least reduce the use of fungicides. There are several valid reasons for eliminating or at least reducing chemical use. Important reasons would include the cost of chemicals, public safety and environmental concerns, and the increase of *S. homoeocarpa* strains resistant to many fungicides. Since many chemical control programs involve applying fungicides every two weeks, this can become quite expensive over a growing season. When it comes to public and environmental safety, many people

are concerned simply from the sight of chemical applications regardless of their danger level. There is also concern about the possibility of these chemicals leaching into water sources. As for fungicide-resistant *S. homoeocarpa*, studies have found strains resistant to the benzimidazole, iprodione, and demethylase inhibitor classes of fungicides (Kane & Miller, 2003; Bishop et al., 2008). Pesticide resistance is a problem that occurs with many pests and usually occurs when the same pesticides are used repeatedly to control these pests. These reasons provide a convincing argument for finding ways to reduce the use of fungicides and thus have led to many studies on the issue.

When attempting to discover methods to reduce the amount of pesticides needed to combat a turfgrass pest, many researchers have examined ways of modifying management practices. Among the typical management practices for creeping bentgrass, one that has been shown to affect dollar spot in multiple studies is the application of nitrogen. As with many other plants, nitrogen is often added to creeping bentgrass to produce more vigorous plants. In addition to this, the application of nitrogen has also been found to decrease dollar spot severity in creeping bentgrass (Williams et al., 1996; Golembiewski and Danneberger, 1998). In most cases though, applications of nitrogen alone are not enough to completely eliminate dollar spot and fungicides are still needed. While nitrogen alone may not control dollar spot at acceptable levels, its positive effects have led to several studies aimed at using it to create alternative control programs that use less chemicals.

A study conducted by Davis and Dernoeden (2002) tested nine different nitrogen sources to determine if some might have a larger effect than others on several factors including dollar spot severity. Among the nitrogen sources tested were several organic

sources as well as synthetics such as urea and sulfur-coated urea. The authors reported that during low disease pressure, a few organic sources were able to reduce dollar spot severity to levels that were comparable to the acceptable levels observed from the use of synthetic sources of N. Unfortunately though, none of the synthetic or organic sources tested were able to provide sufficient dollar spot control during periods of high disease pressure. A similar study conducted by Lee et al. (2003) examined the effects of organic fertilizers and acibenzolar-S-methyl, a plant defense activator, on dollar spot severity. The authors hypothesized that the use of organic fertilizers and/or acibenzolar-s-methyl might possibly reduce the amount of fungicide applications needed to maintain dollar spot to acceptable levels when a fairly dollar spot resistant bentgrass cultivar was used. The authors reported that acibenzolar-s-methyl only significantly reduced dollar spot on fairly susceptible cultivars, but had little effect on 'L-93', the more resistant variety tested. This led the authors to conclude that the use of acibenzolar-s-methyl with organic fertilizers could possibly be integrated in future dollar spot control programs but would not eliminate the need for fungicides.

Golembiewski and Danneberger (1998) examined combining trinexapac-ethyl applications, nitrogen applications, and a blend of 'Crenshaw' and 'Penncross' cultivars to reduce dollar spot severity. The blend of cultivars did not reduce dollar spot severity over Crenshaw alone, but trinexapac-ethyl alone and when combined with nitrogen provided significant dollar spot control over the untreated controls. A high rate of nitrogen alone provided better control than trinexapac-ethyl alone but the high rate of nitrogen combined with trinexapac-ethyl provided the highest reduction of dollar spot

severity. The authors concluded that a combination of trinexapac-ethyl and nitrogen could help to decrease fungicide use for controlling dollar spot on bentgrass.

In addition to the nitrogen fertilizer studies, Zhang et al. (2006) conducted a study to examine if calcium silicate fertilizer applications might decrease dollar spot or brown patch severity. Silicon has been shown to reduce the severity of some other turfgrass and crop diseases (Seebold et al., 2000; Brecht et al., 2004). The authors did not find any significant differences between calcium silicate-treated plots and untreated plots in regard to dollar spot severity.

The nitrogen and fertilizer studies mentioned thus far have dealt with trying to reduce *S. homoeocarpa* damage by making the turf healthier. A healthier bentgrass that is better able to combat a disease on its own should theoretically be less dependent on fungicides. While applying something to the turf that enhances the grass itself has been found to be an effective way of combating a disease in some cases, there are several other ways of attempting to combat a disease as well. Researchers have studied ways to directly attack a pathogen or interfere with the pathogen's environment and survival.

Han et al. (2005) conducted a study that tested the application of a biological control agent known as *Pseudomonas aureofaciens* strain TX-1, which had been shown to affect dollar spot in a laboratory experiment. The authors discovered the agent did reduce dollar spot severity in the field, but not at levels adequate to eliminate the need for fungicides. They concluded that the system could be used with other dollar spot control practices to help reduce severity. The McDonald et al. (2006) study previously mentioned also examined using irrigation timing and amounts to attempt to reduce dollar spot severity. Their study tested the effect of applying a small amount of water every

night to the turf versus applying a larger amount of water to the turf only on mornings when it appeared to need it. The authors found dollar spot severity was worse on the plots that were infrequently irrigated in the morning than on plots that were frequently irrigated at night. Several possible reasons for this were given such as low soil moisture potentially favoring the disease and also the infrequently irrigated plots may have had less mineralization occurring and thus less nitrogen available to the turf to combat the disease. Other studies have shown irrigation to impact early morning dew on the turf which directly affects the dollar spot disease.

As stated earlier, *S. homoeocarpa* mycelium is very evident on diseased turf during periods of heavy dew. Williams et al. (1996) examined dollar spot and dew together by conducting an experiment on the effect of removing dew on dollar spot severity. Dew removal occurred by individual AM mowing and AM poling treatments seven days per week. They reported that removing dew from turf by mowing or poling in the morning resulted in a significant reduction in dollar spot severity with a maximum reduction of 81% on fairway height turf and a 53% reduction on putting green height turf. Williams et al. (1996) also examined the effects of clipping removal on dollar spot severity. By removing clippings some disease-causing components may be reduced (Beard, 1973). It was found though, that there was no significant difference in dollar spot disease severity between leaving and removing clippings. A later study conducted by Williams. (1998) examined the diurnal accumulation rates of dew and re-accumulation rates after dew had been removed. They reported that removal of dew prior to 0400 h generally resulted in significant re-accumulations before sunrise, and recommended that dew removal be accomplished between 0400 and 0600 h.

Ellram et al. (2007) conducted experiments to further test the results of the Williams et al. (1996) work. They examined the effects of several mowing practices on dollar spot severity. Among the variables tested were using a sharp or dull mower blade, mowing everyday or mowing every other day and using a squeegee on non-mowing days, and mowing timing. The authors found that mower blade sharpness had no significant effect on severity, mowing everyday was more effective than mowing every other day and using a squeegee on the other days, and that of the times tested, mowing at 0400 h resulted in the least amount of diseased area. This experiment also examined the effect of leaf wetness duration on dollar spot severity and the authors found that as the amount of time the leaves were wet increased, the severity of dollar spot increased.

Koh et al. (2003) tested the effect of shade and airflow restriction on several characteristics of bentgrass and on dollar spot severity. On many golf courses trees, buildings, and/or other permanent fixtures may reduce the amount of light and/or airflow the turf receives. Decreased irradiance and airflow would not only affect the bentgrass but the environment of the pathogen as well. The authors found that bentgrass plots with airflow restriction typically had higher dollar spot severity than plots in shade or regular airflow conditions. Shaded plots typically had the lowest levels of dollar spot when compared to the airflow restricted and control plots. The authors reported less dew was found in the shade and that it quickly dried without airflow restrictions. The observation of less dew present in the shaded plots, which had less dollar spot, corresponds well to the previously mentioned research studies correlating leaf wetness (dew) and disease severity.

Resistance

Although the previously described experiments have provided some promising alternatives to fungicide use for controlling dollar spot, no research to date has really established a way to completely eliminate the need for fungicides. A possible effective alternative to these control methods though, is host plant resistance. When referring to combating dollar spot, Bonos et al. (2003) wrote “The most promising of the control strategies available is genetic resistance”. Many commercially available cultivars of creeping bentgrass vary in their resistance to dollar spot with some being very susceptible and others fairly resistant. No commercially available cultivar is completely resistant at this time (Morris, 2007). This has led to many experiments examining the mechanisms of resistance and searching for ways to improve it.

When trying to improve a characteristic of a plant, such as its resistance to a disease, many researches begin by experimenting with traditional breeding methods. Turfgrass breeders have had success with improving the disease resistance of several grasses using conventional breeding techniques. Bonos et al. (2004a) reported success in improving resistance to gray leaf spot [*Pyricularia grisea* (Cooke) Sacc. (syn. *P. oryzae* Cavara)] of perennial ryegrass (*Lolium perenne* L.). Fraser and Rose-Fricker (2001) registered ‘Endeavor’ tall fescue (*Festuca arundinacea* Schreb.), which they reported had improved brown patch (*Rhizoctonia solani*) resistance. Several studies have been published that examined ways to breed creeping bentgrass plants that were more resistant to dollar spot and still retained many of the desirable characteristics of a turfgrass such as good color, texture, and density.

Bonos et al. (2003) conducted a study examining dollar spot resistance in terms of heritability and selection. The study used various replicated creeping bentgrass clones and found that heritability was increased and that resistance could be inherited. When using replicated clones and selecting for dollar spot resistance, the authors concluded data should be collected over several years and locations. A study conducted by Chakraborty et al. (2006) used quantitative trait loci (QTL) analysis to attempt to examine genomic regions related to dollar spot resistance in creeping bentgrass. The authors found as Bonos et al. (2003) did that resistance can be inherited, and they were able to locate eight important QTL, one of which had a large effect and seven of which had smaller effects. Information from these two studies can assist breeders in the future in creating bentgrass germplasms with higher resistance to dollar spot.

Belanger et al. (2004) examined how crosses between creeping bentgrass and colonial bentgrass (*Agrostis capillaries* L.) performed in terms of dollar spot resistance. Colonial bentgrass is more resistant to dollar spot than is creeping bentgrass, but the latter is more widely used in America due to its desirable qualities and wider adaptability. In this study, hybrids were created that had improved dollar spot resistance when compared to the creeping bentgrass parents. This led the authors to conclude that the gene(s) responsible for resistance can be transferred but it is unknown if the improved resistance comes from general plant characteristics associated with colonial bentgrass or from specific resistance genes.

In addition to the studies that have examined using traditional breeding to improve dollar spot resistance, other studies have been conducted using genetic engineering. The area of genetic engineering is very large and the study options are

nearly limitless. As with traditional breeding, genetic engineering is conducted in an attempt to improve a plant in some way. In contrast to traditional breeding though, genetic engineering uses laboratory gene-transfer technologies to insert a specific gene or set of genes into a plant instead of making crosses which can transfer many genes. The aim is to hopefully have more control over what is transferred to a plant but unintended or unpredicted effects can still occur in some situations (Cellini et al., 2004). If transferring a gene successfully yields the desired result(s), genetic engineering can often be used to achieve what would have taken a long time or perhaps been impossible with traditional breeding methods. Several studies have been conducted to determine how adding certain genes or proteins to creeping bentgrass cultivars affect their resistance to dollar spot.

Wang et al. (2003) conducted a study to determine the effects of co-transferring a chitinase and glucanase gene into bentgrass with a BAR gene as the selection marker. In the past the co-transfer of these genes in other plants had resulted in improved disease resistance. For this study though, the authors found that the genes themselves did not prevent or reduce disease incidence. Resistance did occur if the plants, which were glufosinate resistant, were sprayed with glufosinate prior to disease inoculation. Another study conducted by Guo et al. (2003) examined how adding the PR5K receptor protein to creeping bentgrass affects its resistance to dollar spot. The PR5K receptor protein has been found to prevent or delay disease progress in lab environments for some plants, but its effect on dollar spot was unknown. For this experiment, transgenic creeping bentgrass plants were created by adding the PR5K protein to embryogenic callus formed from Crenshaw seeds. Of the eight transgenic lines created for the study, four had delayed

dollar spot symptoms and thus the authors conclude PR5K could play a part in attempting to create better dollar spot resistance in creeping bentgrass.

In addition to the previously mentioned studies on creating transgenic and hybrid bentgrass plants, a few other studies on resistance have been conducted that examined how differences in specific turfgrass characteristics can affect resistance. Bonos et al. (2004b) examined the effects of various turf characteristics such as cover, density, stomata density, and trichomes on dollar spot resistance. The authors felt that since factors involved in dollar spot resistance are not completely known or understood, it may be possible that increases or decreases in any of the characteristics listed above could have an effect on resistance. Of the characteristics studied, the authors found that the only one that was significantly correlated with resistance was trichome size. They found that plants with larger trichomes were typically more resistant to dollar spot than plants with smaller trichomes. Trichome numbers were not found to have a significant effect on resistance. Williams and Harrell (2005) conducted a similar study but did not find any significant correlations between the characteristics they examined and dollar spot severity. Trichome size was not a test factor in their study. Dacosta and Ebdon (2008) conducted a study examining how cell wall components as well as nitrogen use and nitrogen reductase activity affected dollar spot resistance in creeping bentgrass. Preliminary results indicated a correlation between dollar spot severity and hemicellulose content with severity being worse in plants with less hemicellulose content. Nitrogen use efficiency did not affect dollar spot severity but reduced nitrate reductase activity was associated with reduced severity.

When examining many of these previous articles, it is often mentioned that the exact mechanism for dollar spot resistance is unknown. Since research thus far has not been successful in defining the mechanism(s) of resistance, it is impossible to predict what they might be. Of the many possible mechanisms, one possible explanation for resistance might be phytoanticipins.

Numerous studies such as ones conducted by Lo et al. (2002) and Pristic et al. (2004) have been conducted examining phytoalexins in grasses and other plants, but much less is known concerning phytoanticipins. When a plant is attacked by a pathogen, antibiotics known as phytoalexins are usually synthesized to combat whatever is invading the plant. In contrast though, some plants may have phytoanticipins. These antibiotics are produced all the time regardless of whether the plant is under attack or not (VanEtten et al., 1994). It is possible phytoanticipins may be present in dollar spot resistant creeping bentgrass plants.

Mert (2006) discussed a group of phytoanticipins known as saponins. The author focused on two saponins known as avenacins and tomatine. Avenacins have been found in oats and break down fungal membranes. Tomatine is found in tomatoes may play a role in combating the pathogen *Cladosporium fulvum* in some varieties. Morrissey and Osbourn (1999) also wrote on anticipins and alexins. In addition to discussing saponins as anticipins, they mentioned several other compounds that might be considered anticipins such as cyanogenic glycosides, glucosinolates, and cyclic hydroxamic acids.

A study conducted by Papadopoulou et al. (1999) examined saponins in oat roots. They were able to create avenacin-deficient mutants and tested how the mutants responded to disease infection. The authors reported that oat plants lacking avenacins

were more susceptible to the *Gaeumannomyces graminis* var. *tritici* fungus than plants that contained avenacins. Roussos et al. (2002) also found some possible anticipins when they conducted a study on rooting enhancements in olive plants brought on by knots formed by *Pseudomonas savastanoi* pv. *savastanoi*. They reported that indole-3-acetonitrile, which had previously been labeled a phytoalexin by past researchers, was present in healthy olive shoots and could possibly be an anticipin.

Objectives

As the previously discussed research indicates, there have been numerous studies aimed at finding ways to reduce dollar spot severity and thus the use of fungicides to control the disease. Field researchers have tested numerous chemicals and management practices in an effort to better control dollar spot and reduce fungicide requirements. Breeders and molecular biologists have examined ways to increase resistance in bentgrass plants through traditional and modern methods. However, no research to date has found a management practice or cultivar that will completely eliminate dollar spot concerns or the need for periodic or regular fungicide applications.

Of the field experiments conducted, one management practice that may provide some promising results is that of dew removal. The dollar spot reduction results Williams et al. (1996) found by removing dew may very well be a possible way to significantly reduce fungicide applications. Concerning traditional plant breeding and molecular genetics research, it is challenging for researchers in these areas to create highly resistant dollar spot plants without knowing the mechanism of dollar spot resistance. If the mechanism(s) was/were known, it could help direct research to locate resistance genes and thus create bentgrass plants that are more resistant to dollar spot than

the currently available cultivars. Based on these two research issues, the following study has two objectives.

The first objective is to determine how many fewer fungicide applications, if any, could be made to adequately minimize dollar spot severity to an acceptable and measureable level when a regular combination dew removal program is used. The second objective is to determine if any antifungal compounds known as phytoanticipins are present in creeping bentgrass germplasms that have shown some level of resistance to dollar spot and if so, if they are an important mechanism of dollar spot resistance.

Chapter Two

Field Studies

Materials and Methods

This experiment was conducted at the University of Kentucky's Spindletop Research Farm and Idlehour Country Club, both in Fayette County, Kentucky. At the Spindletop Farm location, the site was a six year old stand of Penncross creeping bentgrass in a root zone constructed to USGA putting green specifications (90% sand, 10% peat mixture). The Idlehour Country Club site consisted of a stand of L-93 creeping bentgrass seeded in 2005 in Maury silt loam (fine, mixed, mesic, typic Paleudalf). The fairway was located on hole #3 of the course. Testing was initiated on 5 May and 6 May, 2008 for the Spindletop and Idlehour sites, respectively, and continued at both sites until 9 September. Both sites were irrigated to prevent any drought stress, and only between 2000h and 2200h to allow for maximum dew accumulation during the night and early morning hours. Plots were mowed at a height of 1.6 cm every Monday, Wednesday, and Friday between 0700 and 0800h at both sites. This is a very normal mowing regime for creeping bentgrass fairways. Nitrogen was applied to the Spindletop farm location in the form of urea (46-0-0) at a rate of 73.2 kg N ha⁻¹ on 14 November, 2007 with no other applications of N occurring during the experiment. At the Idlehour site, 48.8 kg N ha⁻¹ was applied using a custom blend of Harrell's fertilizer (Harrell's Fertilizer, Inc., Lakeland, FL) which contained 93% Nutralene (18-4-24) in April, 2008. Also, Floratine Largo (12-0-0) (Floratine, Collierville, TN) was applied at the Idlehour site every three weeks throughout the summer at a rate of 6.4 L ha⁻¹, which resulted in 1 kg N ha⁻¹ with each application.

The experimental design for both sites was a 2x3 (dew removal x fungicide) factorial in a randomized complete block with three replications. Plots at both locations were 1.8 x 3 m with 3.0 m and 1.5 m between replications at the Spindletop and Idlehour sites, respectively. Treatments consisted of one of two dew treatments, labeled combination removal (mower-hose; MH) or mower removal (M), combined with one of three fungicide treatments, labeled no fungicides (NF), biweekly applications (BW) or curative applications (C). Plots having the MH treatment had dew removed by mowing three times per week as described above, and by dragging by hose on Sunday, Tuesday, Thursday, and Saturday. Dragging was accomplished by hand-dragging a piece of water hose, 1.8 m in length and 1.6 cm in diameter across the turf. The hose was filled with water to add mass and the ends were capped. Due to the distance between the two testing locations, dragging by hose occurred at 0730h and 0800h for the Spindletop and Idlehour sites, respectively. Plots having the M treatment only had dew removed by mowing Monday, Wednesday, and Friday as previously described with no dragging occurring on the other days. Again, this is the normal mowing/dew removal that creeping bentgrass fairways receive.

All fungicides were applied as commercially available formulations and the application rates provided are expressed as amounts of formulated products. Plots receiving the NF treatment did not receive any fungicide applications for dollar spot during the experiments. Biweekly plots were standard preventative applications made every two weeks, beginning 5 May for the Spindletop site and 6 May for the Idlehour site, using alternating tank-mixes of fosetyl-Al at 12.2 kg ha⁻¹ and iprodione at 12.7 L ha⁻¹ followed by fosetyl-Al 12.2 kg ha⁻¹ and chlorothalonil at 9.76 kg ha⁻¹ (Vincelli and

Powell, 2007). Curative applications were made using the fosetyl-Al and iprodione tank mix at the above rates when dollar spot severity reached a threshold limit within a dew treatment at each location. For the Spindletop site, C applications were made when mean severity was $\geq 20\%$ within a dew treatment. At the Idlehour site, C applications were made when the mean number of active dollar spot centers within a dew treatment was ≥ 10 . In addition to these treatments, azoxystrobin was applied at 1.22 kg ha^{-1} to all plots every three weeks for the duration of the studies to control all other diseases so only dollar spot was present. All applications were made using a CO_2 sprayer with four Tee-Jet #8004 spray tips at a pressure of 207 kPa and a carrier rate of 486 L ha^{-1} .

The response variables were visual estimations of disease severity (percent plot area affected) at the Spindletop location and counts of the number of active dollar spot centers per plot at the Idlehour site. Data were recorded once per week beginning 23 May and ending 9 September for Spindletop and 1 July through 9 September for Idlehour. The delayed start date at Idlehour was due to a lack of visual disease activity prior to the first rating in July.

Data were analyzed using SAS (SAS Institute, 2002-2003). The PROC GLM command was used to separate means through F-protected Fisher's least significant difference (LSD) test ($p \leq 0.05$ at $\alpha = 0.05$).

Results

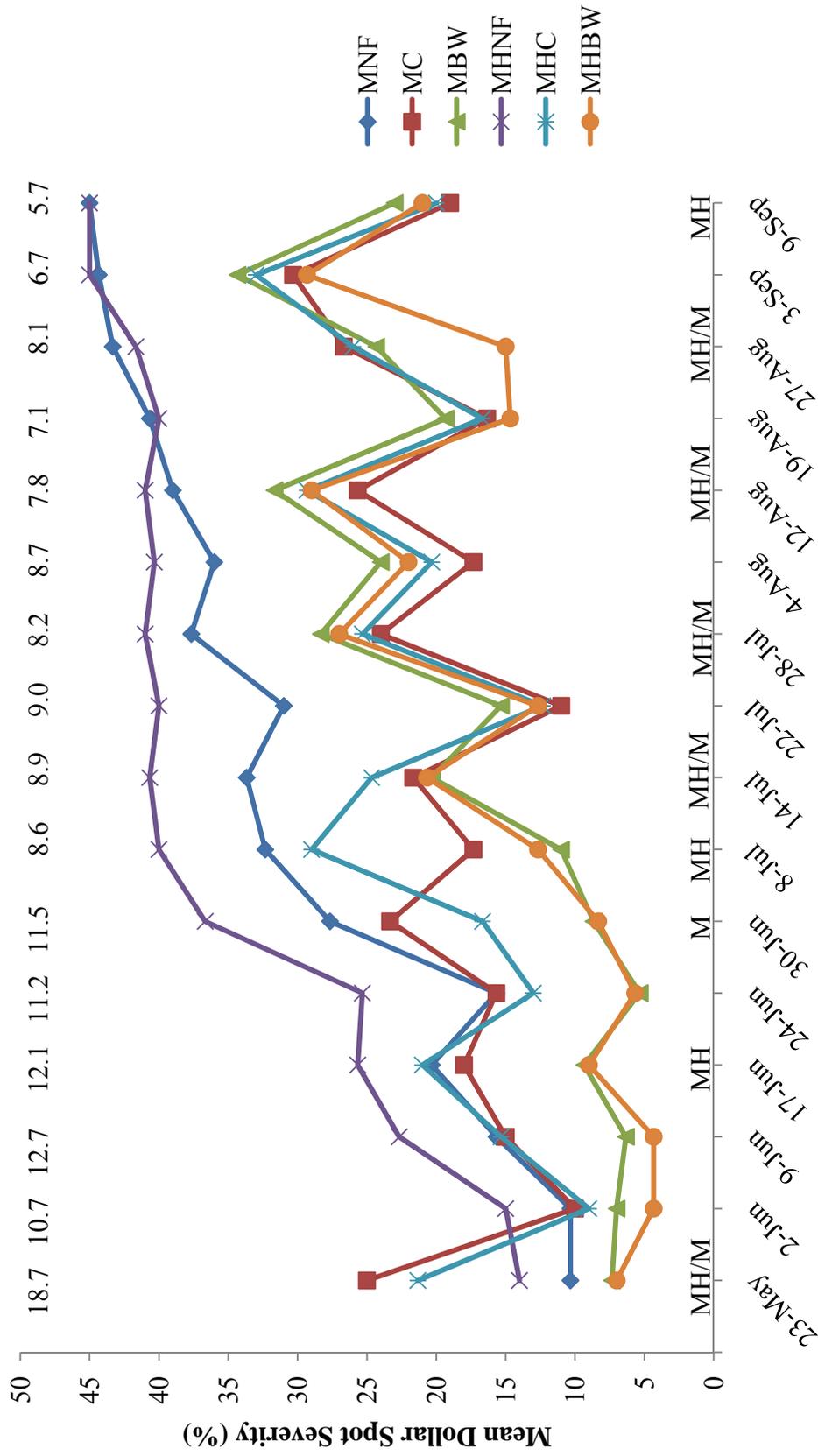
Spindletop Farm

When the experiment was initiated in May, dollar spot was already visible on several plots. Although mean severity was low at first, plots receiving the C treatment were already at the 20% limit by the first observation date of 23 May for both dew

treatments (Fig. 2.1). Disease progress and severity across all treatments increased as the season progressed. Reductions in severity for C and BW treatments throughout the study corresponded directly to fungicide applications.

The main effect of dew treatments was not significant ($P>0.05$) on any date except 8 July (Table 2.1). On that date the main effect of dew treatments was significant ($P<0.05$), with the mean severity for the MH treatment being significantly higher than the mean severity for the M treatment (Table 2.1, Fig. 2.2). For all other dates, disease progress curves were very similar for both dew treatments. Mean severity for the MH treatment ranged from 9.4 to 35.8 % throughout the season and from 9.1 to 36.3 % for the M treatment.

Fungicide treatments expressed more variability. Since disease progress was initially slower at the beginning than later in the season, mean disease severity between NF and BW treatments was not statistically different until the third observation date. By 9 June, and for the remainder of the season, the main effect of fungicide application was significant ($P<0.05$) and mean severity of the NF treatments was significantly higher than the BW treatments (Table 2.1, Fig. 2.3). Mean severity of the C treatments was intermediate compared to the NF and BW fungicide treatments from 2 June to 14 July. By 14 July, severity for C treatments was close to, and not statistically different from BW treatments on all remaining dates except 27 Aug. This is illustrated by disease progress curves for the BW and C treatments which remained very similar from 14 July through the rest of the season (Fig. 2.3). Dips in curves correspond to fungicide applications.



Observation Dates

Figure 2.1. Disease progress curves of dollar spot at the Spindletop site in 2008. Numbers above curves represent F-protected Fisher's LSD values ($p=0.05$). Letters above observation dates indicate curative (C) fungicide applications for the normal mowing (M) and mower-hose (MH) treatments. Biweekly and no fungicide treatments are denoted BW and NF, respectively.

Table 2.1. Analysis of variance values for main effects and potential interactions of dew removal and fungicide treatments at the Spindletop Farm site in 2008.

Source	Observation Date											
	5/23			6/2			6/9			6/17		
	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F
Block (Rep)	0.85	0.46	1.76	0.22	1.99	0.19	3.81	0.0588				
Dew	0.00	0.98	0.01	0.91	0.29	0.60	0.72	0.42				
Fungicide (Fung)	3.79	0.0597	2.12	0.17	6.24	0.02	7.01	0.01				
Dew x Fung	0.19	0.83	0.64	0.55	0.67	0.53	0.27	0.77				
CV (%)	72.7		63.5		52.8		38.6					
		6/24		6/30		7/8		7/14				
Block (Rep)	1.07	0.38	1.97	0.19	3.96	0.0541	1.24	0.33				
Dew	0.71	0.42	0.05	0.83	9.88	0.01	2.23	0.17				
Fungicide (Fung)	9.05	0.01	20.91	0.0003	39.85	<.0001	20.13	0.0003				
Dew x Fung	1.65	0.24	2.32	0.15	1.70	0.23	0.71	0.52				
CV (%)	45.7		31.3		19.9		18.1					

Table 2.1 (Cont.). Analysis of variance values for main effects and potential interactions of dew removal and fungicide treatments at the Spindletop Farm site in 2008.

Source	Observation Date													
	7/22		7/28		8/4		8/12		8/19		8/27		9/9	
	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F
Block (Rep)	1.04	0.39	0.74	0.50	1.15	0.36	0.02	0.98						
Dew	1.20	0.30	0.27	0.61	0.62	0.45	0.25	0.63						
Fungicide (Fung)	42.24	<.0001	17.67	0.0005	27.28	<.0001	14.07	0.0012						
Dew x Fung	2.15	0.17	0.40	0.68	0.73	0.50	0.88	0.44						
CV (%)	24.3		14.8		17.9		13.1							
Block (Rep)	0.11	0.90	2.56	0.13	0.40	0.68	7.84	0.01						
Dew	0.81	0.39	3.47	0.09	0.10	0.75	0.05	0.83						
Fungicide (Fung)	72.31	<.0001	42.13	<.0001	24.93	0.0001	118.95	<.0001						
Dew x Fung	0.68	0.53	1.72	0.23	1.77	0.22	0.35	0.71						
CV (%)	15.9		15.0		10.1		10.9							

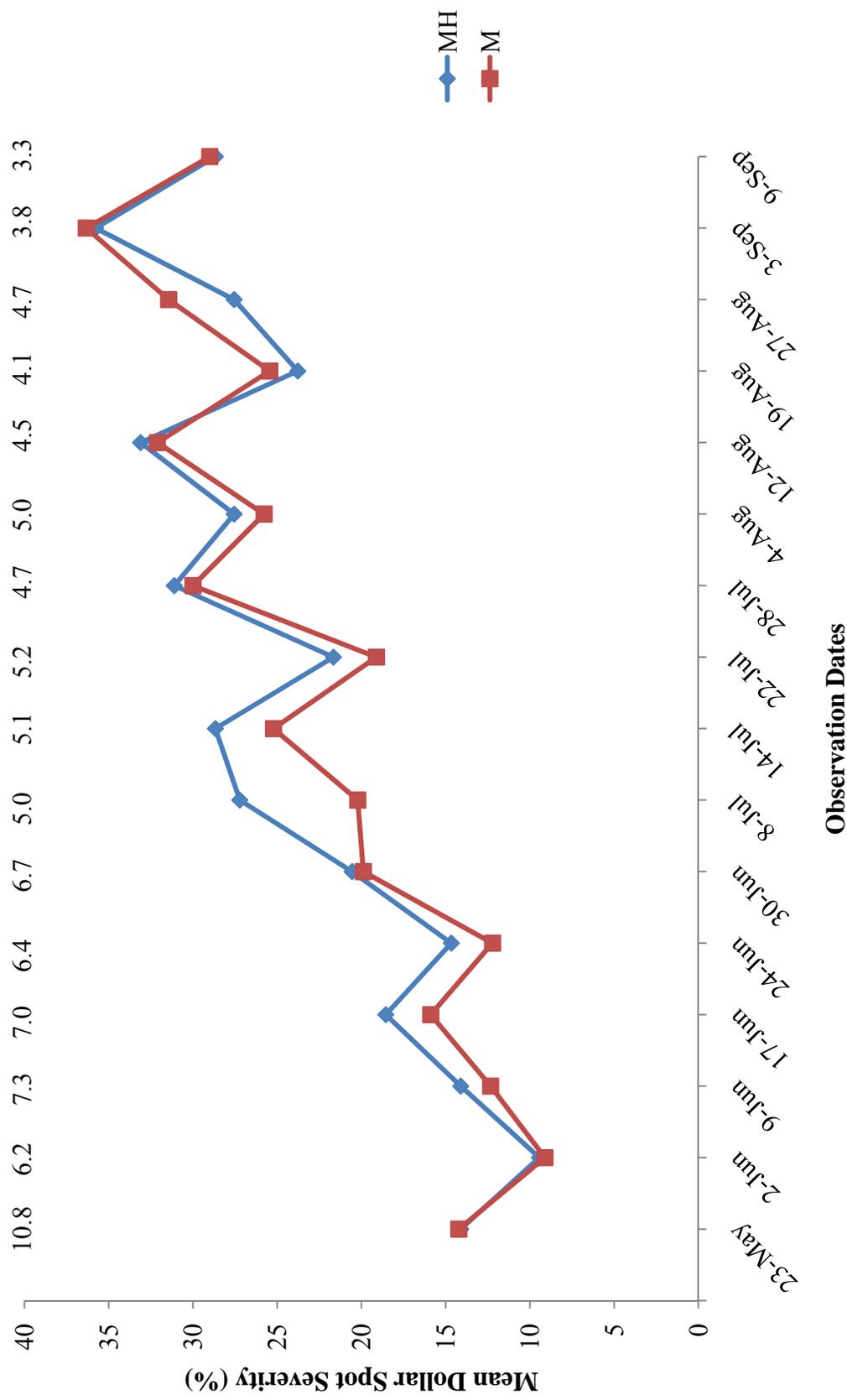


Figure 2.2. Disease progress curves of dollar spot in response to dew treatments at the Spindletop Farm site in 2008. Numbers above curves represent F-protected Fisher's LSD values ($p=0.05$). Mower-hose and mower treatments are denoted MH and M, respectively.

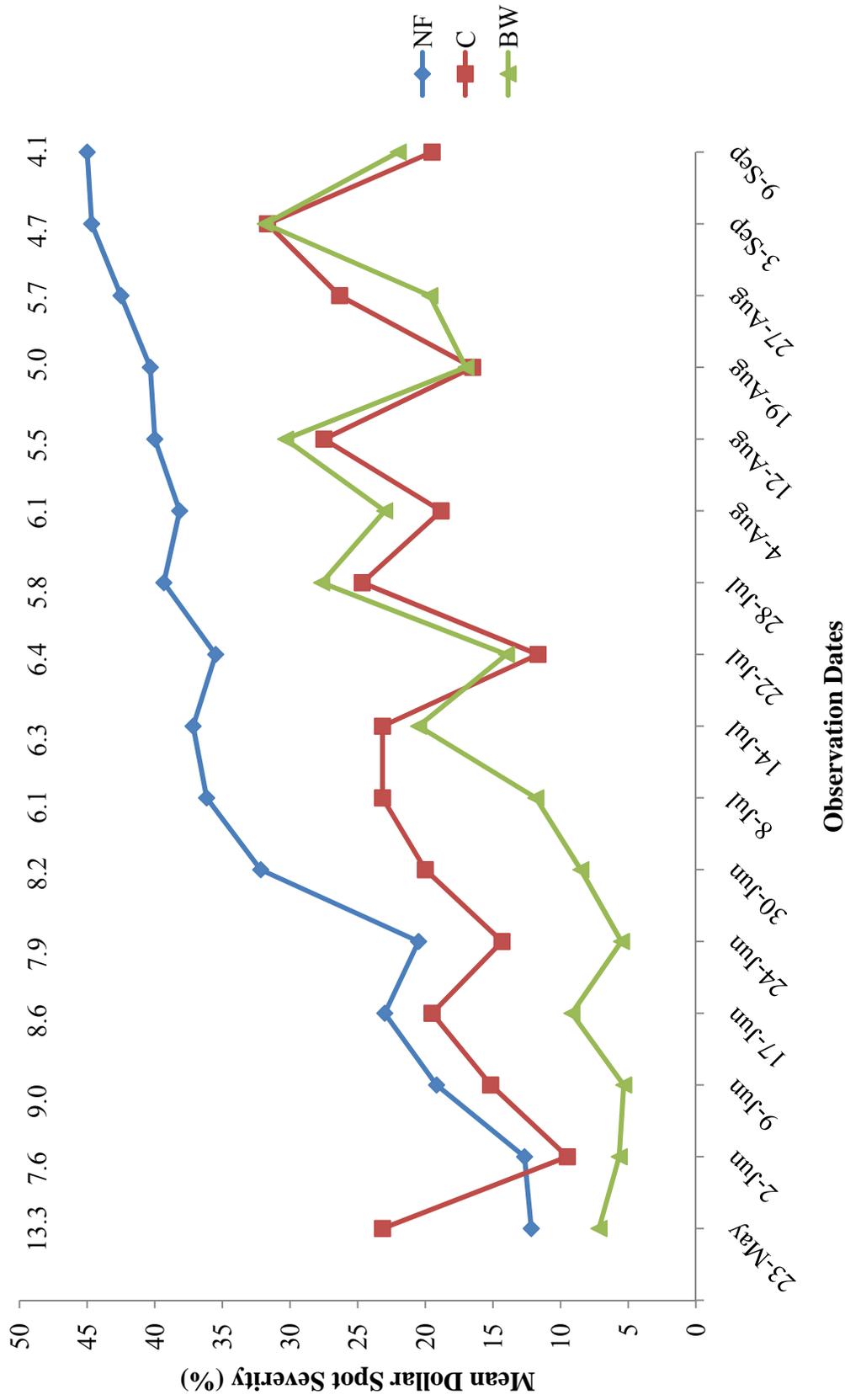


Figure 2.3. Disease progress curves of dollar spot in response to fungicide treatments at the Spindletop Farm site in 2008. Numbers above curves represent F-protected Fisher's LSD values ($p=0.05$). No fungicide, curative, and biweekly treatments are denoted NF, C, and BW, respectively.

Mean severity for individual MH-C and M-C treatments was similar for most of the season although MH-C plots reached the 20% limit twice more than M-C plots which corresponded to two extra fungicide applications on MH-C plots (Fig. 2.1). No dew x fungicide interactions were found to be significant on any date ($P>0.05$) (Table 2.1).

Idlehour Country Club

Disease progress at the Idlehour location was very slow with dollar spot not appearing until late June into early July. Mean severity did not rise above 10 infection centers per plot for any treatment until 5 Aug (Fig. 2.4). For the remainder of the season, disease progress was more rapid except when fungicides were applied to C and BW treatments (Fig. 2.4).

The main effect of dew treatments was not significant ($P>0.05$) on any date (Table 2.2). Mean disease severity across both dew treatments was very close throughout the entire season with a range of 1.1 to 28.7 infection centers for the MH treatment and 1.6 to 31.3 centers for the M treatment (Fig. 2.5).

The main effect of fungicide applications was significant ($P<0.05$) on all dates except 23 July (Table 2.2). Mean severity for NF treatments was statistically different and higher than BW treatments on all dates except 23 July. The C fungicide treatment was not statistically different from NF treatments until 12 Aug. From 12 Aug through the remainder of the season, mean dollar spot progress and severity for the C treatments was less than that of the NF treatment (Fig. 2.6). From 12 Aug through 9 Sep, mean severity for the C treatment was close to severity for the BW treatment and was only statistically different on 12 Aug and 2 Sep. Mean severity for individual C treatments, MH-C and

M-C, was similar for the entire season and each treatment received the same number of fungicide applications (Fig. 2.4). No dew x fungicide interactions were found to be significant on any date ($P>0.05$) (Table 2.2).

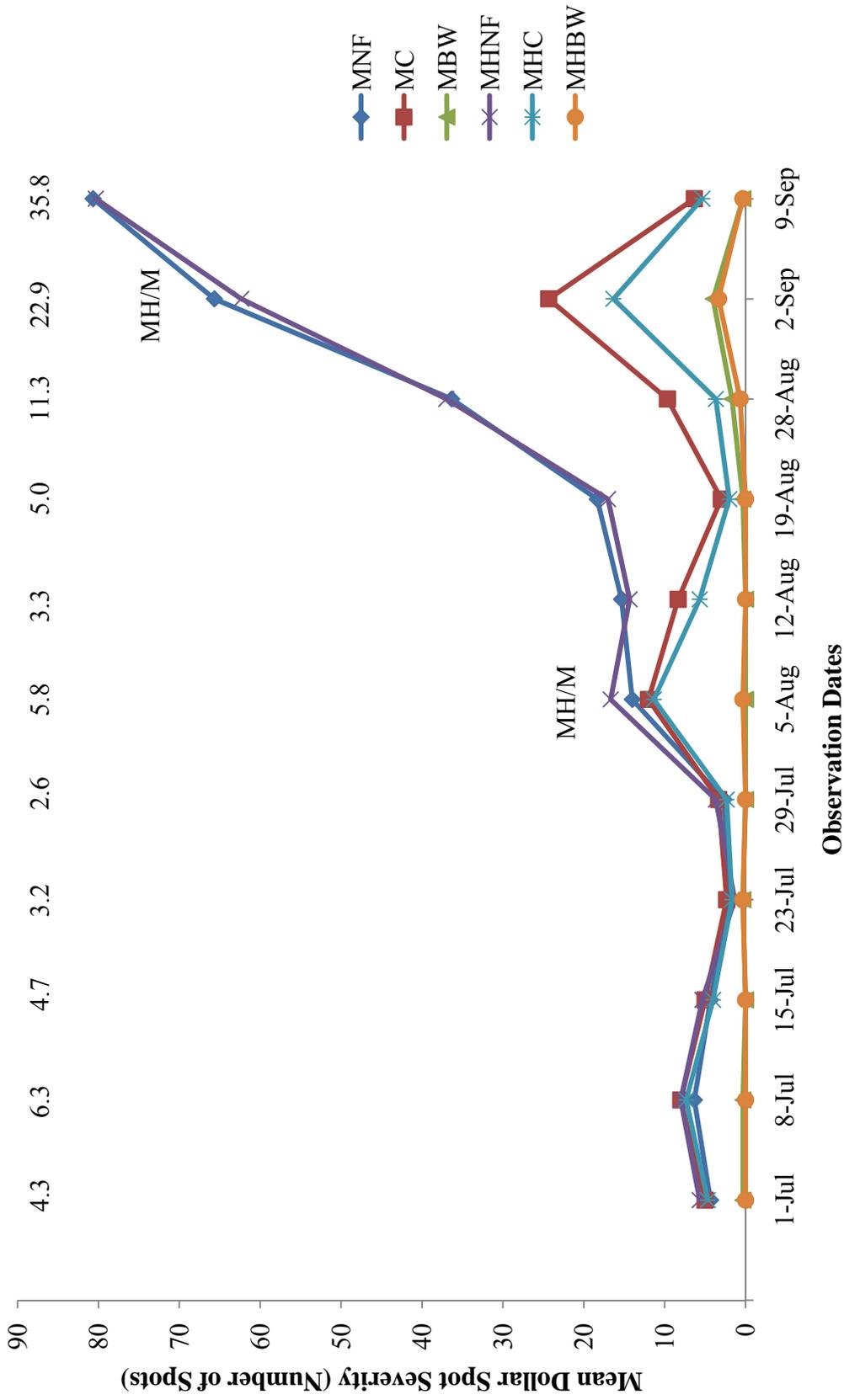


Figure 2.4. Disease progress curves of dollar spot at the Idlehour site in 2008. Numbers above curves represent F-protected Fisher's LSD values ($p=0.05$). Letters above dates indicate curative (C) fungicide applications for the normal mowing (M) and mower-hose (MH) treatments. Biweekly and no fungicide treatments are denoted BW and NF, respectively.

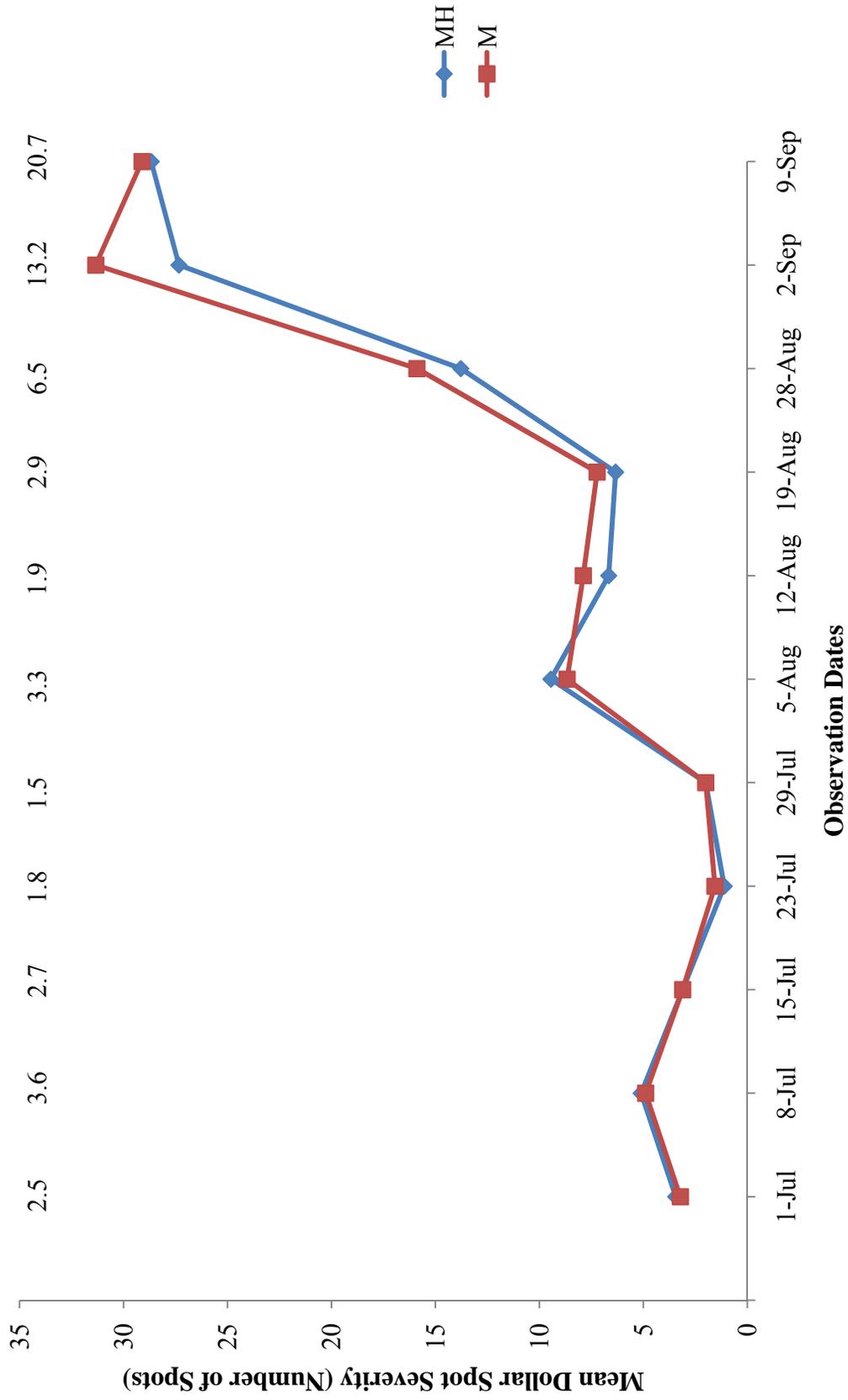


Figure 2.5. Disease progress curves of dollar spot in response to dew treatments at the Idlehour Country Club site in 2008. Numbers above curves represent F-protected Fisher's LSD values ($p=0.05$). Mower-hose and mower treatments are denoted MH and M, respectively.

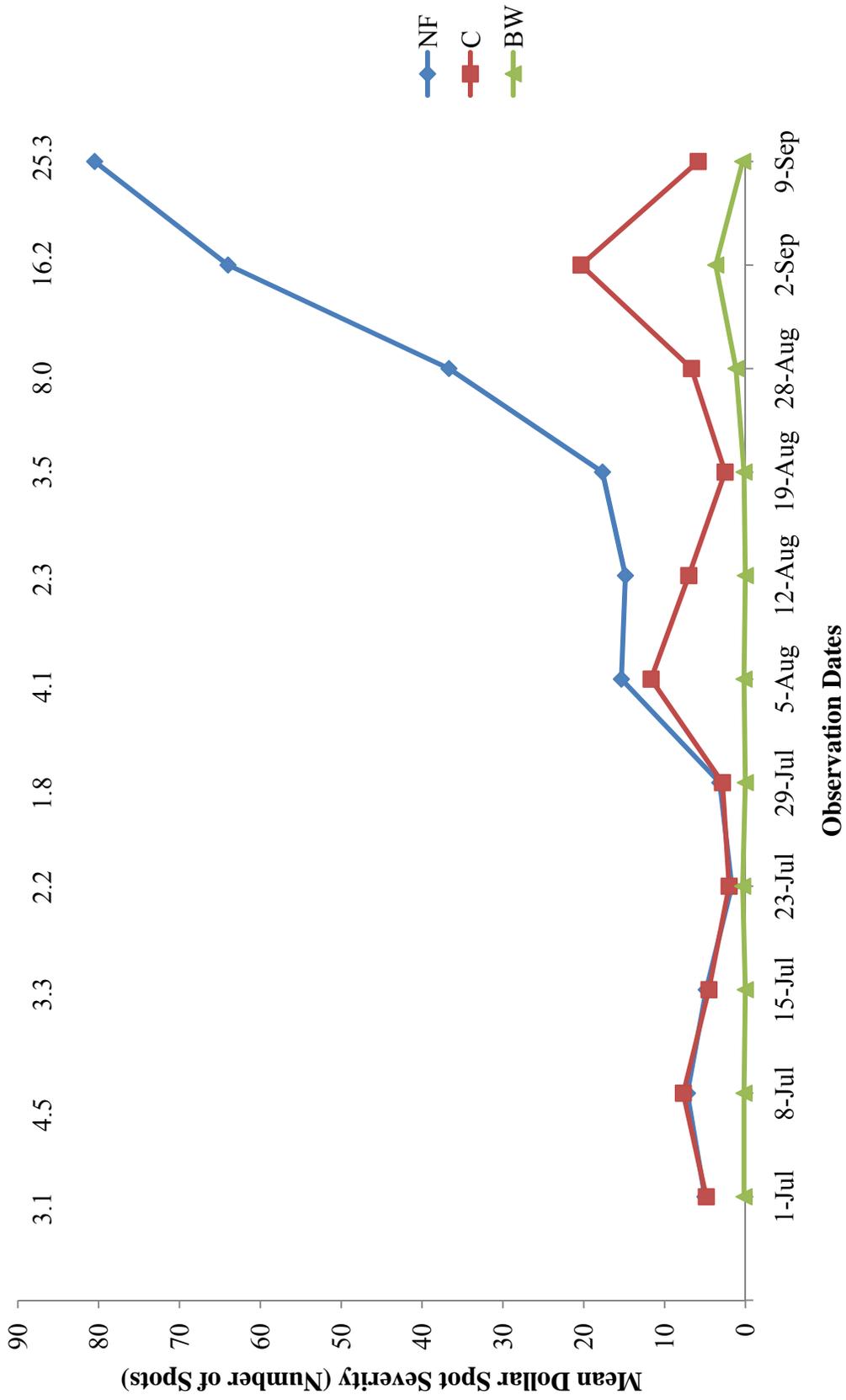


Figure 2.6. Disease progress curves of dollar spot in response to fungicide treatments at the Idlehour Country Club site in 2008. Numbers above curves represent F-protected Fisher's LSD values ($p=0.05$). No fungicide, curative, and biweekly treatments are denoted NF, C, and BW, respectively.

Chapter Three

Phytoanticipin Studies

Materials and Methods

Greenhouse Protocols

One to 2 grams of seed from two experimental lines of creeping bentgrass were obtained from Rutgers University in January, 2008. One line, designated H05F-486 EPC-20 (hereinafter abbreviated R), was considered as being resistant to dollar spot, receiving a mean rating of 8.0 on a visual scale of 1-9 with 9 being highly resistant in 2007 field observations (Bonos, 2008; personal communication). The other line, designated H05F-488 EPC-22 (hereinafter abbreviated S), was considered as being susceptible to dollar spot, receiving a mean rating of 3.0 on the scale in 2007 (Bonos, 2008; personal communication).

The lines were established in a greenhouse on the University of Kentucky's Spindletop Research Farm in March 2008. To establish the lines, a 288-cell tobacco float bed tray was used. The tray was cut in half forming two 144 cell trays. Each tray was filled with Pro-Mix greenhouse medium (Premier Horticulture Inc., Quakertown, PA). Seed from the two experimental lines R and S was evenly distributed by hand across all cells of one tray each. Following seeding, the two trays were placed into separate clear plastic 26.5 L containers which contained approximately 22.7 L of a nutrient solution. Pro-Sol 20-10-20 water soluble fertilizer (Frit Industries, Inc., Ozark, AL) was dissolved at a rate of 0.75g/L to create the nutrient solution. White lids with a cut-out square slightly larger than the size of the trays were placed onto the plastic containers to prevent the trays from moving in the nutrient solution. Once the trays were in place, an air hose

with a diffuser tip connected to a small aquarium pump was inserted into the solution of each container to provide adequate oxygenation of the nutrient solution.

The greenhouse was maintained at a temperature of 18 to 21 °C. No grow lamps were used so all light was provided by natural sunlight. Additional nutrient solution (also at 0.75g/L) was added when the level in the containers began to drop as a result of evaporation and root uptake. Once the plants reached several centimeters in height, they were trimmed with scissors or electric clippers every Monday, Wednesday, and Friday to approximately 2.5 cm.

Trays were periodically examined for root development. When root length reached several centimeters in length and began touching the bottom of the plastic containers, 236 mL samples of the nutrient solutions were collected from each container, labeled appropriately, and frozen. Approximately one week later, a white cottony growth began covering the float bed trays indicative of a potential pythium blight outbreak (*Pythium sp.*). At this time, 473 mL samples of the nutrient solutions from each container were collected and frozen. Hereinafter, the nutrient solution samples are referred to as early (E) and late (L) representing the first and second collections described above, respectively. At the same time as the L sample of the nutrient solution was collected from a container, the plugs from each individual cell of the tray were harvested. The top growth was separated from the roots and each were placed in plastic bags, labeled, and frozen immediately.

Solvent Extraction

Testing of the nutrient solution samples began in January 2009 with chloroform, hexane, and ethyl acetate extractions of the L samples. To begin the extractions, three 50

mL samples from the R and three samples from the S nutrient solutions were poured into six separate 50 mL plastic tubes. Also, three 50 mL samples of a fresh nutrient mixture alone and three samples of fresh nutrient solution injected with 30 μ L of capsidiol, a known antifungal compound, were poured into six additional separate plastic 50 mL tubes. The nutrient solutions with (C) and without (NC) capsidiol acted as positive and negative controls.

The chloroform extraction procedure for each sample was as follows. An individual 50 mL nutrient solution sample was poured into a separatory funnel along with 50 mL of chloroform. A stopper was placed onto the funnel, it was then inverted, the stopcock opened, and the funnel was shaken for 15 to 20 seconds. The stopcock was then closed, the funnel uprighted, the stopper was removed, and the funnel was allowed to sit for 1-2 minutes. After this time, the lower phase was drained from the funnel into a collection flask. Then an additional 50 mL of chloroform was added to the funnel and the procedure repeated with the lower phase being collected again and the upper phase then being discarded after this second extraction. This entire procedure was repeated for the hexane and ethyl acetate extractions of the samples with the only difference being that the top phase of these two solvents was collected instead of the lower phase as with chloroform.

Once a sample had been extracted, the collection flask was evaporated to dryness using a rotoevaporator. After a sample dried, it was resuspended twice in 250 μ L of the original extraction solvents. The samples were transferred from the collection flasks into autosampler vials. To concentrate the samples, the vials were dried down with nitrogen

and re-suspended in 50 μL of the extraction solvent. After this step, the vials were placed into a refrigerator.

TLC Analysis

Samples were initially analyzed using thin layer chromatography (TLC). Twelve dots, corresponding to the twelve samples (1 sample each of R, S, C, and NC extracted in each of the 3 solvents), spaced 0.75 cm apart and 1.5 cm from the bottom of the plate were drawn on the TLC plate. Each dot was labeled and 5 μL of each sample was applied by syringe to its respective dot. The plate was then developed in approximately 40 mL of cyclohexane:acetone (1:1). Once the solvent front had moved close to the top of the TLC plate, the plate was removed and allowed to air dry. After the plate had dried, it was visually evaluated under UV light for possible fluorescence from one or several of the compounds of interest (Papadopoulou et al., 1999). The plate was then sprayed to saturation with an indicator reagent composed of 1.4 g vanillin, 40 mL methanol, and 250 μL concentrated sulfuric acid. After the plate was saturated from the indicator reagent it was dried with a hair dryer and visually evaluated for a color reaction to the indicator reagent for the compounds of interest.

New Extractions, Second TLC Analysis, and Bioassay

Based on the results from the first run of the experiment described above, new chloroform extractions were conducted on 50 mL samples of C and NC solutions, and samples of the R-E and S-E solutions and R-L and S-L solutions from the greenhouse. This constituted a total of six 50 mL samples. The extraction procedure for these samples was the same as previously described.

Once the six samples had been resuspended and concentrated to 50 μL , they were analyzed on two new TLC plates. The TLC procedure was the same as previously described except instead of applying the 5 μL from the samples as one small dot, the 5 μL was applied over a line between two dots spaced 1.5 cm apart. After the two plates had developed and dried, one plate was sprayed with the indicator reagent and dried while the other plate was sprayed with a *Cladosporium cucumerinum* spore suspension. The TLC plate sprayed with the indicator reagent was visually evaluated and the other plate sprayed with *C. cucumerinum* was placed in a dark, moist environment and visually evaluated for fungal growth several days later.

New Plants

In order to replicate the aforementioned TLC analyses and to potentially refine the protocol, new R and S plants were established and grown in the greenhouse. The establishment protocol for these plants was similar to the original protocol but some changes were made. Two trays of each line were grown instead of one to provide more nutrient solution and plant material for analyses. In an effort to concentrate the nutrient solutions, the trays were placed in new, smaller plastic containers. The new containers were approximately half the size of the original containers and were spray painted black on the outside in an attempt to minimize algae development. Tops were not placed on the new containers since the smaller size prevented the trays from moving. Also, these plants were grown in a greenhouse on the University of Kentucky's campus. This greenhouse was maintained at a temperature of 25 °C during the day and 21 °C at night and had supplemental lighting. The supplemental lighting was automatically initiated from 0600h to 2000h when the ambient photosynthetically active radiation (PAR) was

below $500 \mu\text{mol m}^{-2} \text{sec}^{-1}$ and automatically ended when ambient PAR reached $600 \mu\text{mol m}^{-2} \text{sec}^{-1}$.

Extraction and Analysis of New Solutions

The new solutions were extracted with chloroform as previously described. Six total samples were used for these extractions which consisted of the C and NC solutions, as well as two 50 mL samples of the R solution and two 50 mL samples of the S solution. After the six samples were extracted, 2 TLC plates were made and developed as previously described; one for TLC analysis and one for bioassay analysis.

Results

TLC Analysis

The first TLC plate, which tested 12 samples, provided several results. First, the UV light used to examine possible fluorescence exposed a solid bar above the resistance line sample extracted in chloroform. A bar also seemed to be present above the standard nutrient solution extracted in ethyl-acetate although this bar was very faint and harder to see than the bar above the resistant sample. No other samples had any visible fluorescence.

The indicator reagent caused several dots to appear on the TLC plate. The capsidiol samples extracted in chloroform and ethyl-acetate each had a dark bluish dot appear approximately 3.5 cm from the initial application spot. A blue dot also appeared approximately 3.5 cm from the initial application spot for the capsidiol sample extracted in hexane, although the dot had a lighter blue color (Fig. 3.1). No other samples initially expressed anything significant. Approximately one hour later the TLC plate was

inspected again and a bluish dot was visible about 4 cm above the initial sample application point for the R sample extracted in chloroform (Fig. 3.2).

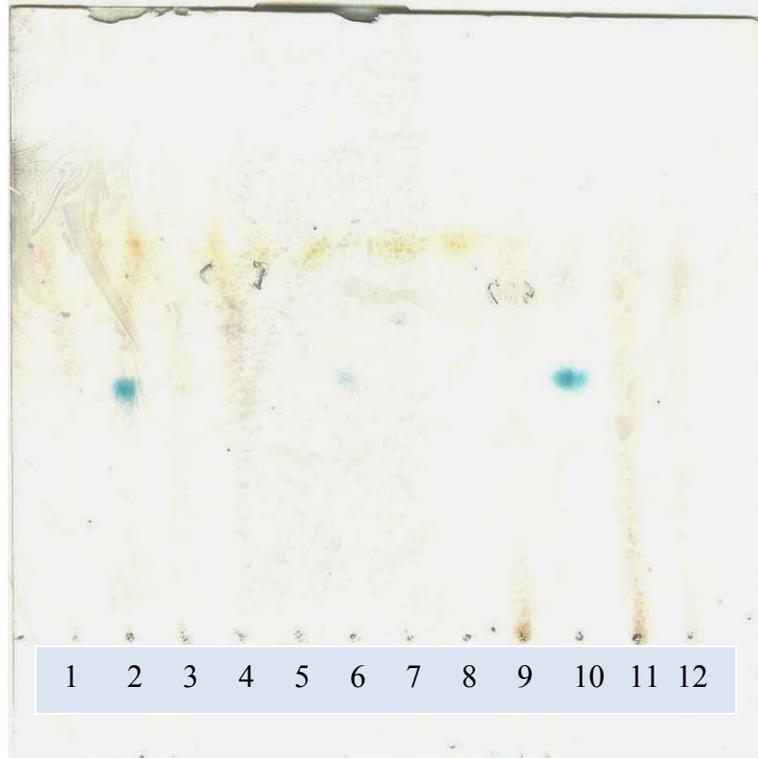
The second TLC analysis of 6 samples expressed different results than the first plate. UV light showed a faint bar above the initial application point for the R-E sample but did not show anything for the R-L sample which came from the same container as the resistant sample that had some fluorescence on the first TLC plate. After applying the indicator reagent and heat to the plate, a very faint bluish line appeared above the initial application point for the capsidiol but nothing appeared for any of the other samples.

The last TLC used to test the two new sets of plants grown in 2009 also provided different results than the initial TLC analysis. UV light did not show any fluorescence for any of the samples. The indicator reagent and heat also did not show any blue lines above the resistant samples or any other samples except for the capsidiol sample which had a blue line approximately 3.5 cm from the initial application point.

Bioassays

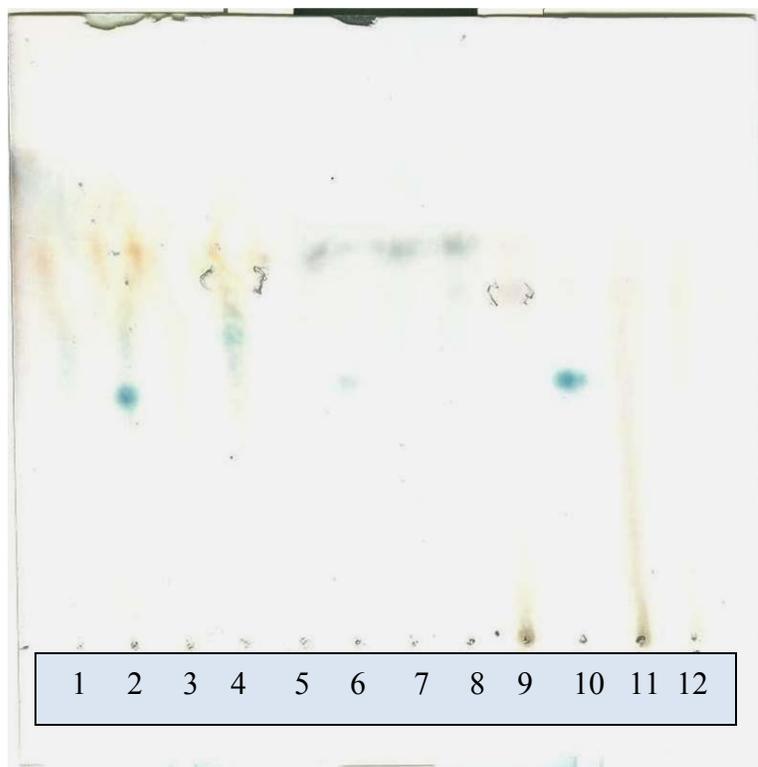
The first bioassay plate, which was of the 6 samples including the R-E and R-L samples, had a good coverage of *Cladosporium* when inspected three to four days after being sprayed with spores. Fungal growth had occurred across the entire plate except approximately 5 cm above the initial application point for the R-E sample (Fig 3.3). Growth above the capsidiol application point may have been slightly less dense than across the other areas of the plate, but it was still covered and an actual void did not occur like above the R-E sample.

The second bioassay plate, which was of the new greenhouse samples, had fairly good *Cladosporium* growth but did not express anything significant by visual evaluation.



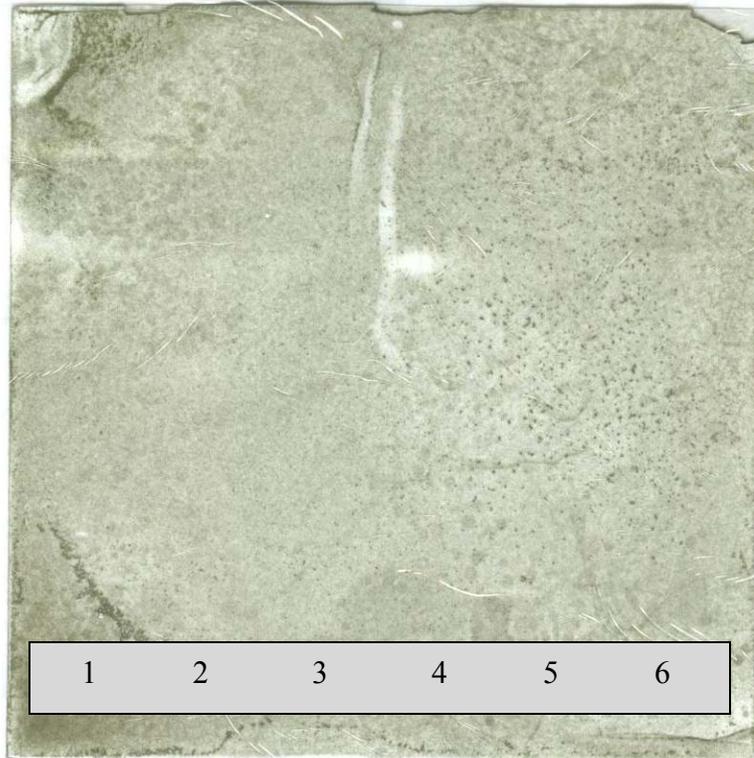
1. No capsidiol sample extracted in chloroform
2. Capsidiol sample extracted in chloroform
3. Susceptible sample extracted in chloroform
4. Resistant sample extracted in chloroform
5. No capsidiol sample extracted in hexane
6. Capsidiol sample extracted in hexane
7. Susceptible sample extracted in hexane
8. Resistant sample extracted in hexane
9. No capsidiol sample extracted in ethyl acetate
10. Capsidiol sample extracted in ethyl acetate
11. Susceptible sample extracted in ethyl acetate
12. Resistant sample extracted in ethyl acetate

Figure 3.1. Initial TLC results for first set of extractions. Samples were applied directly to dots above numbers.



1. No capsidiol sample extracted in chloroform
2. Capsidiol sample extracted in chloroform
3. Susceptible sample extracted in chloroform
4. Resistant sample extracted in chloroform
5. No capsidiol sample extracted in hexane
6. Capsidiol sample extracted in hexane
7. Susceptible sample extracted in hexane
8. Resistant sample extracted in hexane
9. No capsidiol sample extracted in ethyl acetate
10. Capsidiol sample extracted in ethyl acetate
11. Susceptible sample extracted in ethyl acetate
12. Resistant sample extracted in ethyl acetate

Figure 3.2. TLC results for first set of extractions approximately one hour later from the initial evaluation. Note appearance of blue dot in lane 4, the resistant sample extracted in chloroform.



1. No capsdiol sample
2. Capsidiol sample
3. Susceptible-early sample
4. Resistant-early sample
5. Susceptible-late sample
6. Resistant-late sample

Figure 3.3. Bioassay results for the second set of extractions. Samples were applied in a straight line with the numbers placed below the mid-point of the lines. Note the possible zone of fungal inhibition in lane 4, the resistant-early sample.

Chapter Four

Discussion and Conclusions

Field Studies

The use of a combination dew removal program consisting of mowing three days per week and dragging by hose on the other four days did not prove to be a successful method for reducing the number of fungicide applications necessary to manage dollar spot disease on creeping bentgrass at acceptable levels. Mean dollar spot severity throughout this study was very close for both dew treatments at both locations with the differences in severity only being significant on one date and at one location. This resulted in the same number of C fungicide applications being made to both dew treatments at the Idlehour Country Club location (Fig. 4.1). There was a difference of two C fungicide applications at the Spindletop Farm site between dew treatments, but the two additional applications were required for the MH treatment instead of the M treatment. Since the main effect of dew treatments was not significant, it can be concluded this difference was based on random variability among plots and was not caused by the dew treatments.

Overall, fewer C fungicide treatments were made throughout the season than BW preventative treatments at both locations (Fig. 4.1). At Spindletop Farm, the maximum reduction in fungicide applications was 40% for the C treatments compared to the normal BW treatments. Idlehour Country Club had an even greater reduction of 80% fewer C treatments compared to BW treatments. While these reductions in fungicide applications may not be attributable to the dew removal treatments, they are nonetheless important to note since they suggest it may be possible to maintain dollar spot at acceptable levels

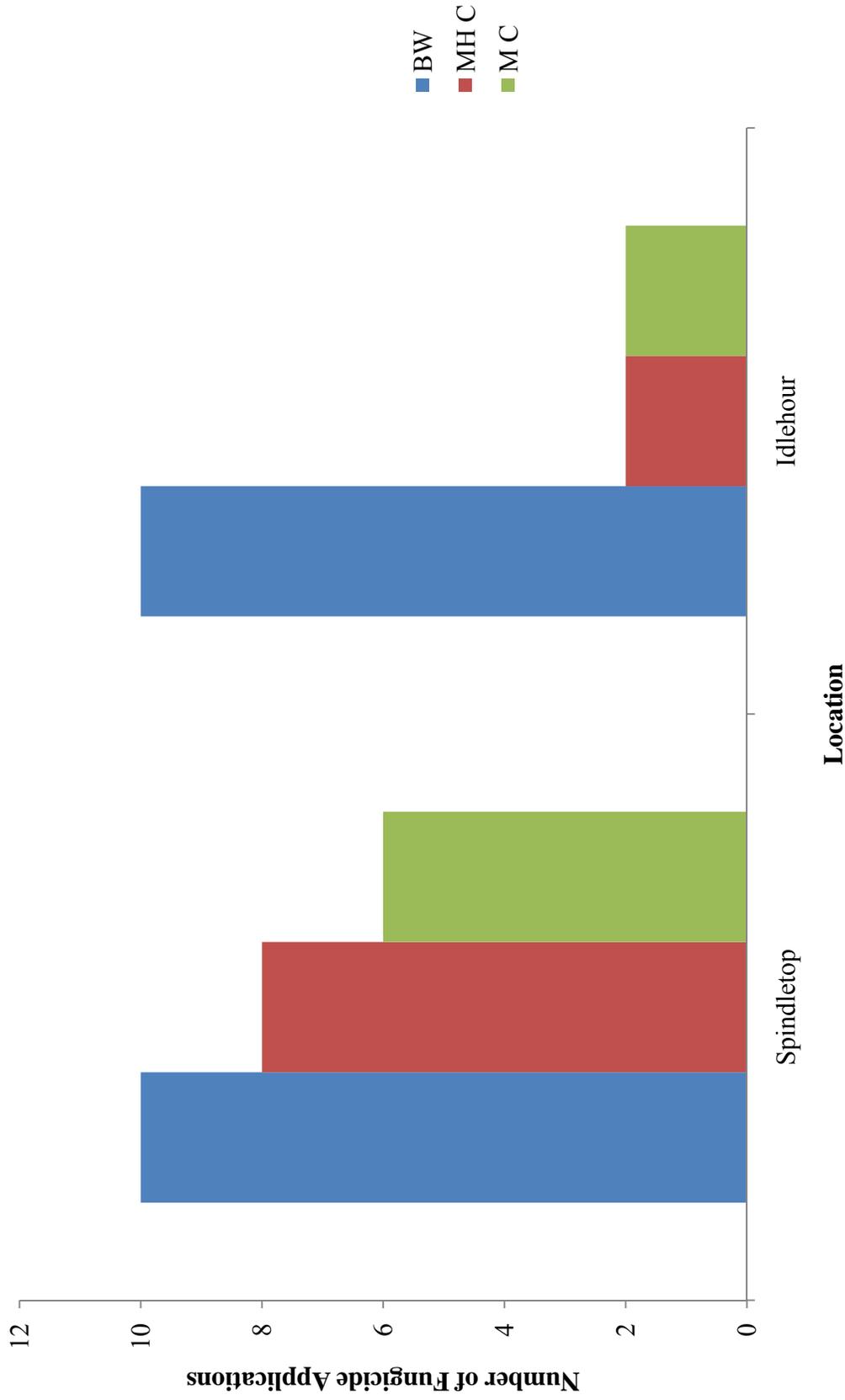


Figure 4.1. Total number of curative (C) and biweekly (BW) fungicide applications made for each of the dew removal treatments at the Spindletop Farm and Idlehour Country Club sites in 2008. Mower-hose and mower treatments are denoted MH and M, respectively.

with fewer chemical applications compared to commonly-used preventative programs.

The lack of measureable effects from the combination dew removal program in this study (mowing and dragging by hose) is in contrast to the results reported by Ellram et al. (2007). Their study found a combination of dew removal methods to be significant in reducing disease severity. It should be noted though that their study involved removing dew by pulling a rigid, rubber floor squeegee on the non-mowing days instead of dragging by hose. Additionally, the levels of disease severity reported in that study were significantly less than disease severity levels in this study. Ellram et al. (2007) also reported, as did Williams et al. (1996), that mowing everyday is by far the most effective strategy of dew removal in terms of reducing dollar spot severity. The results found in this study and by Ellram et al. (2007) and Williams et al. (1996) seem to suggest the forceful removal of dew through methods such as mowing five to seven days a week is the most successful method to reduce dollar spot severity and potentially reduce chemical use. Unfortunately, it is not practical to mow golf courses fairways at this frequency.

Future research should further investigate the chemical reductions observed when fungicides are sprayed curatively instead of preventatively. It is currently not known what level of disease severity would be acceptable to the general public on golf course fairways, but if a threshold limit could be established (e.g., the 10 spots per plot used at the Idlehour site), it may be possible to drastically reduce the fungicide requirements for controlling dollar spot using a curative strategy similar to the regime used in this study. If a general threshold were established, researchers could examine the number of fungicide applications needed to maintain this threshold on various cultivars managed under various conditions, and then compare this number of applications with typical

preventative programs. It is this researcher's opinion that while using a curative fungicide program to control dollar spot instead of a preventative program may not consistently provide the 40-80% maximum reductions observed in this study, it would most likely result in fewer fungicide applications in most years on even the more disease susceptible cultivars. The use of more resistant cultivars would probably result in fewer fungicide applications still.

Phytoanticipin Studies

Concerning the presence or absence of phytoanticipins in creeping bentgrass, firm conclusions cannot be drawn at this time based on the variability of results observed among TLC and bioassay plates. However, there were results that provoke further consideration and investigation. Since the resistant line extracted in chloroform produced fluorescence under UV light and eventually expressed a bluish dot on the TLC plate when nothing appeared from the NC and S samples, it suggests there may have been compounds in the R sample that were not present in the other samples. On the bioassay plate, the void over the R-E sample indicates possible inhibition of fungal growth, perhaps caused by a plant-generated compound which could potentially be extracted and identified. The lack of fungal growth suppression, or at least significant suppression, over the capsidiol application point would indicate the potential antifungal compound(s) in the R-E sample is/are more effective than capsidiol. For the other TLC plates, no differences were observed for the R samples when compared to the S samples other than the slight fluorescence of the R-E sample on the second plate under UV light. Also, the second bioassay plate did not express any fungal growth inhibition for the R samples. It should be noted though that the second bioassay plate did not have any inhibition above

the capsidiol application point either which indicates potential problems with the protocol; i.e., the preparation or development of that plate. It is also of importance to note that the application methods for samples on the second and third TLC plate were different from that of the first. On the first plate, all 5 μ L samples were applied only to one small dot whereas on the second and third plates, the 5 μ L samples were applied evenly over a 1.5 cm line. This would decrease the concentration of the sample at any one point. If the concentration of significant compounds in the R samples was low to begin with, this could make delineating them more difficult using this particular protocol.

Since these results are preliminary, and may suggest the presence of compounds in the R samples that are not in the S samples, there is adequate evidence to warrant further investigation. Future studies should work to refine the protocol and test other bentgrass germplasms possessing varying degrees of disease resistance. While analysis of the nutrient solutions used to grow the plants was important, tissue analyses (both root and shoot) must also be conducted since it may be possible that antifungal compounds could be produced and sequestered *in planta*. The results of Bonos et al. (2004b) showing trichome size to be potentially related to disease resistance also calls for additional research. The trichomes could potentially be glandular in which case they may release compounds that could inhibit fungal growth.

Summary

Research aimed at reducing chemical applications to combat dollar spot must continue since public and environmental safety concerns as well as fungicide costs are increasing. While I am not sure that much more can currently be tested with dew removal effects on disease severity, other results such as the ones presented in this work

provide promising future possibilities. The 20 to 40% reduction in chemical use observed at the Spindletop Farm site was certainly positive, but the 80% reduction of fungicide applications on a more disease resistant cultivar at the Idlehour site is highly significant. Also, while it is difficult to determine if the 20% disease severity limit used at the Spindletop Farm site is acceptable, the ten infection spots per plot threshold at Idlehour would probably be considered acceptable when as much as 80% fewer fungicide applications can be made throughout a season and still maintain that level of disease control. In addition to these results, other work such as by McDonald et al. (2006) and Fidanza et al. (2006) suggests the need to further examine the effects of other management practices. For example, the application of PGRs along with nitrogen source, amount, and frequency should be investigated for their effect on potentially reducing dollar spot severity. Again, the use of highly susceptible versus more resistant bentgrass germplasms in these studies would aid in quantifying the effects of these and other management practices.

While results from phytoanticipin studies were not immediately conclusive, TLC plates as well as a bioassay plate did indicate potential differences between the R and S lines. Therefore, additional research in this area could potentially yield highly significant results. Defining the mechanism(s) of resistance would be a major step in working to improve dollar spot disease resistance and ultimately reducing fungicide use. Even if phytoanticipins are not found in creeping bentgrass, it will at least eliminate one possibility and help narrow down the search for the true resistance mechanisms and eventually help lead to their discovery.

Appendix A

Spindletop Farm Treatment Means

Table A.1. Mean disease severity† of dollar spot across all treatment combinations at the Spindletop Farm site in 2008.

<u>Treatment</u>	<u>Observation Date</u>								
	<u>5/23</u>	<u>6/2</u>	<u>6/9</u>	<u>6/17</u>	<u>6/24</u>	<u>6/30</u>	<u>7/8</u>	<u>7/14</u>	
Mower, Bi-weekly	7.3a	7.0a	6.3b	9.3b	5.3b	8.7c	11.0c	20.3b	
Mower, Curative	25.0a	10.0a	15.0ab	18.0ab	15.7ab	23.3b	17.3c	21.7b	
Mower, No Fungicides	10.3a	10.3a	15.7ab	20.3ab	15.7ab	27.7ab	32.3ab	33.7a	
Mower-Hose, Bi-weekly	7.0a	4.3a	4.3b	9.0b	5.7b	8.3c	12.7c	20.7b	
Mower-Hose, Curative	21.3a	9.0a	15.3ab	21.0ab	13.0b	16.7bc	29.0b	24.7b	
Mower-Hose, No Fungicides	14.0a	15.0a	22.7a	25.7a	25.3a	36.7a	40.0a	40.7a	
	<u>7/22</u>	<u>7/28</u>	<u>8/4</u>	<u>8/12</u>	<u>8/19</u>	<u>8/27</u>	<u>9/3</u>	<u>9/9</u>	
Mower, Bi-weekly	15.3b	28.3b	24.0b	31.7bc	19.3b	24.3b	34.3b	23.0b	
Mower, Curative	11.0b	24.0b	17.3b	25.7c	16.3b	26.7b	30.3b	19.0b	
Mower, No Fungicides	31.0a	37.7a	36.0a	39.0ab	40.7a	43.3a	44.3a	45.0a	
Mower-Hose, Bi-weekly	12.7b	27.0b	22.0b	29.0c	14.7b	15.0c	29.3b	21.0b	
Mower-Hose, Curative	12.3b	25.3b	20.3b	29.3c	16.7b	26.0b	33.0b	20.0b	
Mower-Hose, No Fungicides	40.0a	41.0a	40.3a	41.0a	40.0a	41.7a	45.0a	45.0a	

†Mean disease severity is a visual estimation of the percent of the plot affected by *Sclerotinia homoeocarpa*.

Table A.2. Mean disease severity† of dollar spot across dew treatments at the Spindletop Farm site in 2008.

<u>Treatment</u>	<u>Observation Date</u>							
	<u>5/23</u>	<u>6/2</u>	<u>6/9</u>	<u>6/17</u>	<u>6/24</u>	<u>6/30</u>	<u>7/8</u>	<u>7/14</u>
Mower-Hose	14.1a	9.4a	14.1a	18.6a	14.7a	20.6a	27.2a	28.7a
Mower	14.2a	9.1a	12.3a	15.9a	12.2a	19.9a	20.2b	25.2a
	<u>7/22</u>	<u>7/28</u>	<u>8/4</u>	<u>8/12</u>	<u>8/19</u>	<u>8/27</u>	<u>9/3</u>	<u>9/9</u>
Mower-Hose	21.7a	31.1a	27.6a	33.1a	23.8a	27.6a	35.8a	28.7a
Mower	19.1a	30.0a	25.8a	32.1a	25.4a	31.4a	36.3a	29.0a

†Mean disease severity is a visual estimation of the percent of the plot affected by *Sclerotinia homoeocarpa*. Means followed by the same letter are not significantly different by F-protected Fisher's LSD test (P>0.05).

Table A.3. Mean disease severity† of dollar spot across fungicide treatments at the Spindletop Farm site in 2008.

<u>Treatment</u>	<u>Observation Date</u>								
	<u>5/23</u>	<u>6/2</u>	<u>6/9</u>	<u>6/17</u>	<u>6/24</u>	<u>6/30</u>	<u>7/8</u>	<u>7/14</u>	
No Fungicides	12.2ab	12.7a	19.2a	23.0a	20.5a	32.2a	36.2a	37.2a	
Biweekly Applications	7.2b	5.7a	5.3b	9.2b	5.5b	8.5c	11.8c	20.5b	
Curative Applications	23.2a	9.5a	15.2a	19.5a	14.3a	20.0b	23.2b	23.2b	
	<u>7/22</u>	<u>7/28</u>	<u>8/4</u>	<u>8/12</u>	<u>8/19</u>	<u>8/27</u>	<u>9/3</u>	<u>9/9</u>	
No Fungicides	35.5a	39.3a	38.2a	40.0a	40.3a	42.5a	44.7a	45.0a	
Biweekly Applications	14.0b	27.7b	23.0b	30.3b	17.0b	19.7c	31.8b	22.0b	
Curative Applications	11.7b	24.7b	18.8b	27.5b	16.5b	26.3b	31.7b	19.5b	

†Mean disease severity is a visual estimation of the percent of the plot affected by *Sclerotinia homoeocarpa*. Means followed by the same letter are not significantly different by F-protected Fisher's LSD test (P>0.05).

Appendix B
Idlehour Country Club Treatment Means

Table B.1. Mean disease severity† of dollar spot across all treatment combinations at the Idlehour Country Club site in 2008.

<u>Treatment</u>	<u>Observation Date</u>										
	<u>7/1</u>	<u>7/8</u>	<u>7/15</u>	<u>7/23</u>	<u>7/29</u>	<u>8/5</u>	<u>8/12</u>	<u>8/19</u>	<u>8/28</u>	<u>9/2</u>	<u>9/9</u>
Mower, Bi-weekly	0.3bc	0.3bc	0.0b	0.3a	0.0b	0.0b	0.0c	0.3b	1.7b	4.0b	0.3b
Mower, Curative	5.0a	8.0a	5.0a	2.3a	3.3a	12.0a	8.3b	3.0b	9.7b	24.3b	6.3b
Mower, No Fungicides	4.3ab	6.3ab	4.3ab	2.0a	2.7a	14.0a	15.3a	18.3a	36.3a	65.7a	80.7a
Mower-Hose, Bi-weekly	0.0c	0.0c	0.0b	0.3a	0.0b	0.3b	0.0c	0.0b	0.7b	3.3b	0.3b
Mower-Hose, Curative	4.7a	7.3a	4.0ab	1.7a	2.3ab	11.3a	5.7b	2.0b	3.7b	16.3b	5.3b
Mower-Hose, No Fungicides	5.7a	8.0a	5.3a	1.3a	3.7a	16.7a	14.3a	17.0a	37.0a	62.3a	80.3a

†Mean disease severity is a visual count of the number of infection centers in each plot caused by *Sclerotinia homoeocarpa*.

Table B.2. Mean disease severity† of dollar spot across dew treatments at the Idlehour Country Club site in 2008.

<u>Treatment</u>	<u>Observation Date</u>										
	<u>7/1</u>	<u>7/8</u>	<u>7/15</u>	<u>7/23</u>	<u>7/29</u>	<u>8/5</u>	<u>8/12</u>	<u>8/19</u>	<u>8/28</u>	<u>9/2</u>	<u>9/9</u>
Mower-Hose	3.4a	5.1a	3.1a	1.1a	2.0a	9.4a	6.7a	6.3a	13.8a	27.3a	28.7a
Mower	3.2a	4.9a	3.1a	1.6a	2.0a	8.7a	7.9a	7.2a	15.9a	31.3a	29.1a

†Mean disease severity is a visual count of the number of infection centers in each plot caused by *Sclerotinia homoeocarpa*. Means followed by the same letter are not significantly different by F-protected Fisher's LSD test (P>0.05).

Table B.3. Mean disease severity† of dollar spot across fungicide treatments at the Idlehour Country Club site in 2008.

<u>Treatment</u>	<u>Observation Date</u>										
	<u>7/1</u>	<u>7/8</u>	<u>7/15</u>	<u>7/23</u>	<u>7/29</u>	<u>8/5</u>	<u>8/12</u>	<u>8/19</u>	<u>8/28</u>	<u>9/2</u>	<u>9/9</u>
No Fungicides	5.0a	7.2a	4.8a	1.7a	3.2a	15.3a	14.8a	17.7a	36.7a	64.0a	80.5a
Biweekly Applications	0.2b	0.2b	0.0b	0.3a	0.0b	0.2b	0.0c	0.2b	1.2b	3.7c	0.3b
Curative Applications	4.8a	7.7a	4.5a	2.0a	2.8a	11.7a	7.0b	2.5b	6.7b	20.3b	5.8b

†Mean disease severity is a visual count of the number of infection centers in each plot caused by *Sclerotinia homoeocarpa*. Means followed by the same letter are not significantly different by F-protected Fisher's LSD test (P>0.05).

References

- Beard, J.B. 1973. Turfgrass: Science and Culture. Prentice-Hall, Englewood Cliffs, NJ.
- Belanger, F.C., S. Bonos, and W.A. Meyer. 2004. Dollar spot resistant hybrids between creeping bentgrass and colonial bentgrass. *Crop Sci.* 44: 581-586.
- Bishop, P., J. Sorochan, B.H. Ownley, T.J. Samples, A.S. Windham, M.T. Windham, and R.N. Trigiano. 2008. Resistance of *Sclerotinia homoeocarpa* to iprodione, propiconazole, and thiophanate-methyl in Tennessee and northern Mississippi. *Crop Sci.* 48: 1615-1620.
- Bonos, S.A., M.D. Casler, and W.A. Meyer. 2003. Inheritance of dollar spot resistance in creeping bentgrass. *Crop Sci.* 43: 2189-2196.
- Bonos, S.A., C. Kubik, B. B. Clarke, and W.A. Meyer. 2004a. Breeding perennial ryegrass for resistance to gray leaf spot. *Crop Sci.* 44: 575-580.
- Bonos, S.A., M.D. Casler, and W.A. Meyer. 2004b. Plant responses and characteristics associated with dollar spot resistance in creeping bentgrass. *Crop Sci.* 44: 1763-1769.
- Brecht, M.O., L.E. Datnoff, T.A. Kucharek, and R.T. Nagata. 2004. Influence of silicon and chlorothalonil on the suppression of gray leaf spot and increase plant growth in St. Augustinegrass. *Plant Dis.* 88: 338-344.
- Burpee, L., and R. Latin. 2008. Reassessment of fungicide synergism for control of dollar spot. *Plant Dis.* 92: 601-606.
- Cellini, F., A. Chesson, I. Colquhoun, A. Constable, H.V. Davies, K.H. Engel, A.M.R. Gatehouse, S. Karenlampi, E.J. Kok, J.-J. Leguay, S. Lehesranta, H.P.J.M. Noteborn, J. Pedersen, and M. Smith. 2004. Unintended effects and their detection in genetically modified crops. *Food Chem. Toxicol.* 42: 1089-1125.
- Chakraborty, N., J. Curley, S. Warnke, M.D. Casler, and G. Jung. 2006. Mapping QTL for dollar spot resistance in creeping bentgrass. *Theor. Appl. Genet.* 113:1421-1435.
- DaCosta, M., and J.S. Ebdon. 2008. Relationship between cell wall components, nitrogen use efficiency and nitrate reductase activity in dollar spot resistance among *Agrostis* species and genotypes. Abstract. 2008 CSSA Annual Meetings Houston, TX.
- Davis, J.G., and P.H. Dernoeden. 2002. Dollar spot severity, tissue nitrogen, and soil microbial activity in bentgrass as influenced by nitrogen source. *Crop Sci.* 42: 480-488.

- Ellram, A., B. Horgan, and B. Hulke. 2007. Mowing strategies and dew removal to minimize dollar spot on creeping bentgrass. *Crop Sci.* 47: 2129-2137.
- Fidanza, M.A., H.C. Wetzel III, M.L. Agnew, J.E. Kaminski. 2006. Evaluation of fungicide and plant growth regulator tank-mix programmes on dollar spot severity of creeping bentgrass. *Crop Prot.* 25: 1032-1038.
- Fraser, M.L., and C.A. Rose-Fricker. 2001. Registration of 'Endeavor' tall fescue. *Crop Sci.* 41:1997.
- Golembiewski, R.C., and T.K. Danneberger. 1998. Dollar spot severity as influenced by trinexapac-ethyl, creeping bentgrass cultivar, and nitrogen fertility. *Agron. J.* 90: 466-470.
- Guo, Z., S. Bonos, W.A. Meyer, P.R. Day, and F.C. Belanger. 2003. Transgenic creeping bentgrass with delayed dollar spot symptoms. *Mol. Breeding* 11: 95-101
- Han, D., J. Rimelspach, B. Parisi, and M. Boehm. 2005. Biological control of dollar spot of golf courses using *Pseudomonas aureofaciens* TX-1 delivered via the BioJect. The Ohio State University Publication. Online.
http://www.ca.uky.edu/agcollege/plantpathology/ext_files/golfcourses.pdf.
 Accessed 5 September 2007.
- Hsiang, T., and S. Cook. 2005. The effect of winter applications of azoxystrobin on dollar spot incidence during the following summer. Guelph Turfgrass Institute 2005 Annual Research Report. Online.
<http://www.uoguelph.ca/GTI/05anrep/82-84.pdf>. Accessed 25 November 2008.
- Kane, R., and L. Miller. 2003. Controlling dollar spot that is resistant to fungicides 2001-2002 data. InteractiveTurf.com. Online.
http://www.interactiveturf.com/Research/research_DS_2003116.htm.
 Accessed 2 January 2008.
- Koh, K.J., G.E. Bell, D.L. Martin, and N.R. Walker. 2003. Shade and airflow restriction effects on creeping bentgrass golf greens. *Crop Sci.* 43: 2182-2188.
- Lee, J., J. Fry, and N. Tisserat. 2003. Dollar spot in four bentgrass cultivars as affected by acidbenzolar-s-methyl and organic fertilizers. *Plant Health Progress*. Online.
<http://www.plantmanagementnetwork.org/pub/php/research/2003/dollar/>.
 Accessed 28 August 2007.
- Lo, C., R.C. Coolbaugh, and R.L. Nicholson. 2002. Molecular characterization and *in silico* expression analysis of a chalcone synthase gene family in *Sorghum bicolor*. *Physiol. Mol. Plant Path.* 61: 179-188.

- McDonald, S.J., P.H. Dernoeden, and C.A. Bigelow. 2006. Dollar spot and gray leaf spot severity as influenced by irrigation, chlorothalonil, paclobutrazol, and a wetting agent. *Crop Sci.* 46: 2675-2684.
- Mert, Figen. 2006. Saponins versus plant fungal pathogens. *J. Cell Mol. Biol.* 5: 13-17.
- Morris, K.N. 2007. Dollar spot ratings of bentgrass cultivars grown on a fairway or tee. Table 24A. 2004-07 Data Final Report NTEP No. 08-7. USDA-ARS. Beltsville, MD. Online. http://www.ntep.org/data/bt03f/bt03f_08-7f/bt03f08ft24a.txt. Accessed 25 February 2009.
- Morrissey, J.P., and A.E. Osbourn. 1999. Fungal resistance to plant antibiotics as a mechanism of pathogenesis. *Microbiol. Mol. Biol. Rev.* 63(3): 708-724.
- Papadopoulou, K., R.E. Melton, M. Leggett, M.J. Daniels, and A.E. Osbourn. 1999. Compromised disease resistance in saponin-deficient plants. *P. Natl. Acad. Sci. USA* 96 (22): 12923-12928.
- Prisic, S., M. Xu, P.R. Wilderman, and R.J. Peters. 2004. Rice contains two disparate *ent*-copalyl diphosphate synthases with distinct metabolic functions. *Plant Physiol.* 136: 4228-4236.
- Roussos, P.A., C.A. Pontikis, and E. Tsantili. 2002. Root promoting compounds detected in olive knot extract in high quantities as a response to infection by the bacterium *Pseudomonas savastanoi* pv. *savastanoi*. *Plant Sci.* 163: 533-541.
- SAS for Windows. 2002-2003. Version 9.1, SAS Inst. Inc., Cary, NC.
- Seebold, K.W., L.E. Datnoff, F.J. Correa-Victoria, T.A. Kucharek, and G.H. Snyder. 2000. Effect of silicon rate and hose resistance on blast, scald, and yield of upland rice. *Plant Dis.* 84: 871-876.
- Smiley, R.W., P.H. Dernoeden, and B.B. Clarke. 2005. Compendium of turfgrass diseases. 3rd ed. American Phytopathological Society. St. Paul, MN.
- VanEtten, H.D., J.W. Mansfield, J.A. Bailey, and E.E. Farmer. 1994. Two classes of plant antibiotics: phytoalexins versus "phytoanticipins". *Plant Cell* 6: 1191-1192.
- Vincelli, P., J.C. Doney, Jr., and A.J. Powell. 1997. Variation among creeping bentgrass cultivars in recovery from epidemics of dollar spot. *Plant Dis.* 81: 99-102.
- Vincelli, P., and A.J. Powell. 2007. Chemical control of turfgrass diseases 2008. University of Kentucky Extension Publication. PPA-1.

- Wang, Y., A.P. Kausch, J.M. Chandlee, H. Luo, B.A. Ruummele, M. Browning, N. Jackson, M.R. Goldsmith. 2003. Co-transfer and expression of chitinase, glucanase, and *bar* genes in creeping bentgrass for conferring fungal disease resistance. *Plant Sci.* 165: 497-506.
- Williams, D.W., A.J. Powell, Jr., P. Vincelli, and C.T. Dougherty. 1996. Dollar spot on bentgrass influenced by displacement of leaf surface moisture, nitrogen, and clipping removal. *Crop Sci.* 36: 1304-1309.
- Williams, D.W., A.J. Powell, Jr., C.T. Dougherty, and P. Vincelli. 1998. Separation and quantitation of the sources of dew on creeping bentgrass. *Crop Sci.* 38: 1613-1617.
- Williams, D.W. and M.S. Harrell. 2005. Leaf surface morphology among *Agrostis* species and cultivars and correlation with dollar spot severity. *USGA Turfgrass and Environmental Research Online* 4(10): 1-6. TGIF Record Number: 105213.
- Zhang, Q., J. Fry, K. Lowe, and N. Tisserat. 2006. Evaluation of calcium silicate for brown patch and dollar spot suppression on turfgrasses. *Crop Sci.* 46: 1635-1643.

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