IMPACT OF IRRIGATION REGIME AND HOST CULTIVAR ON DOLLAR SPOT OF CREEPING BENTGRASS

By

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A THESIS

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ABSTRACT

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Dollar spot (DS), caused by *Sclerotinia homoeocarpa*, is an important disease of turfgrass. Irrigation practices and host resistance can impact disease incidence and aesthetics on fairway turfgrass. This study was conducted to determine the impact of irrigation regime and creeping bentgrass cultivar on DS incidence as well as vegetative compatibility group (VCG) of the pathogen. Irrigation was applied at either 2200 h daily or twice weekly, or 0500 h daily to three creeping bentgrass cultivars, 'Declaration', 'SRP-1WM', and 'L-93'. 'Declaration' and 'SRP-1WM' were considered resistant to DS and 'L-93' was considered susceptible. Plots watered at 2200 h daily exhibited significantly less disease than those irrigated at 2200 h twice weekly, regardless of creeping bentgrass cultivar. 'SRP-1WM' developed the least amount of DS each year among all cultivars. In 2011 and 2013, the 'SRP-1WM' plots receiving daily irrigation (AM or PM) did not significantly differ and exhibited less DS than those irrigated at 2200 h twice weekly for the same cultivar.

Recovered isolates of *S. homoeocarpa* were scored for VCG based on barrage zone formation to determine if irrigation regime or creeping bentgrass cultivar corresponded uniquely to VCG. Results indicated that VCGs B and F, averaging 45 and 33% overall, respectively, were the most predominant pairing regardless of irrigation regime or host cultivar. These results indicate that specific irrigation regimes or host cultivars investigated in this study similarly influenced VCG. To my husband, Mark For your endless love and support

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CHAPTER I:

IMPACT OF IRRIGATION REGIME AND CREEPING BENTGRASS CULTIVAR ON DOLLAR SPOT ON FAIRWAY TURF IN MICHIGAN

Abstract

Irrigation regime and cultivar selection can impact disease incidence on fairway turfgrass. Improvements in dollar spot (Sclerotinia homoeocarpa FT Bennett) resistance of various cultivars of creeping bentgrass (Agrostis stolonifera L.) have been achieved based on breeding efforts. An integrated approach to dollar spot management has been attained by combining irrigation regime and host cultivar. Three irrigation regimes in combination with three creeping bentgrass cultivars were studied for interactive impacts on dollar spot development over three years. Irrigation was applied in one of three programs: 0500 h daily, 2200 h daily, or 2200 h twice weekly. Total seasonal irrigation applications were within 1 cm for all irrigation treatments and were calculated and calibrated to deliver approximately equivalent amounts of water weekly. The cultivars of creeping bentgrass tested were 'Declaration' and 'SRP-1WM', considered resistant cultivars, and 'L-93', a susceptible cultivar. Relative area under the disease progress curve (RAUDPC) was calculated from dollar spot incidence data each year. In 2012, dollar spot pressure was extremely low and no conclusions were made using this data. However, in 2011 and 2013, plots irrigated at 2200 h daily exhibited significantly less dollar spot than those irrigated at 2200 h twice weekly, regardless of cultivar of creeping bentgrass. The least amount of dollar spot developed on 'SRP-1WM' in each year of the trial. In 2011 and 2013, the 'SRP-1WM' plots receiving daily irrigation (0500 or 2200 h) were not significantly different from each other at p = 0.05 but exhibited less dollar spot than those receiving the 2200 h twice weekly program. The 2200 h daily irrigation program resulted in less dollar spot than the 2200 h twice weekly irrigation program when approximately equivalent amounts of water were applied weekly.

Introduction

Water is vital to life and consumption is continually scrutinized and even regulated through legislation in some parts of the USA (Cisar et al., 2004). Restrictions for recreational use of water, such as for turf management, have resulted from these regulations (Southwest Florida: www.swfwmd.state.fl.us/conservation/restrictions/; Texas: www.tceq.texas.gov/drinkingwater/trot/droughtw.html; Sanderson, 2013). Approximately 80% of maintained turfgrass on golf courses in the USA is irrigated, where nearly 100% of greens, tees, and fairways are irrigated (Throssell et al., 2009). Various conservation efforts are often voluntarily implemented by turfgrass managers, including improvements to irrigation systems (Lyman et al., 2007). Water management is just one facet of the complex of strategies used to maintain amenity turf. Other daily practices include mowing. Pest management is also of great concern for turf managers, often consuming large portions of maintenance budgets (Vargas, 2005).

Dollar spot is a plant disease found worldwide on most turfgrass species (Smiley et al., 2005) and is caused by the fungus *Sclerotinia homoeocarpa* FT Bennett, although its taxonomy is under contention (Kohn, 1979; Whetzel, 1945; Baldwin and Newell, 1992). Dollar spot is considered to be one of the most economically important diseases on amenity turf due to costs associated with control (Vargas, 2005).

Symptoms of dollar spot in stands of turfgrass include blighted, circular spots ranging in size up to 5 cm in diameter on fairways and putting greens, or 15 cm on athletic fields or home lawns. The disease was named "dollar spot" due to the resemblance of the spots on putting greens and fairways to the size of a silver dollar (Bennett, 1937). Spots are often sunken and can coalesce, developing into larger

irregular-shaped areas (Smiley et al., 2005). White, cottony mycelia may be visible under environmental conditions conducive to development of dollar spot and especially in the presence of dew. On leaf blades, dollar spot lesions become chlorotic, watersoaked and turn white to tan-colored, with a reddish-brown perimeter in species other than *Poa annua* (Vargas, 2005). Lesions can occur transversely across a leaf or may spread longitudinally from the apex of the leaf.

Dollar spot is prevalent from late spring through the end of fall in the northern USA (Vargas, 2005). The pathogen spreads by direct contact with neighboring plants and by dissemination of infected clippings via equipment such as mowers, and by humans and animals (Allen et al., 2005). The aerial mycelia infect the leaf on contact if the host is susceptible and environmental conditions are conducive. The fungus survives periods of unfavorable conditions as mycelia in infected plant tissue and debris or as stromata. Infection occurs during periods of high humidity in the turf canopy with temperatures typically between 15-30°C. Dollar spot is often worse under conditions of low nitrogen fertility and soil moisture levels below field capacity (Smiley et al., 2005; Vargas, 2005; Walsh et al., 1999).

Management of dollar spot is accomplished through the use of chemical, cultural, biological, and genetic means. Although chemical management is among the most common practices to reduce dollar spot, integrated management practices are frequently employed (Vargas, 2005; Watkins et al., 2001). Successful dollar spot management occurs with a combination of cultural practices and fungicide usage. Biological control of dollar spot has been attained through the use of commercially available products containing antimicrobial agents (biofungicides), however, biological

control products tend to be most successful under conditions of low to moderate disease severity (Smiley et al., 2005; Vargas, 2005; Walsh et al., 1999). Commercially available cultivars of creeping bentgrass (Agrostis stolonifera L.) have been developed that are less susceptible to dollar spot as compared to older cultivars (NTEP, 2010; Belanger et al., 2004; Bonos, 2006; Bonos et al., 2006). Because infection of plants by S. homoeocarpa occurs under conditions where relative humidity exceeds 85% in the canopy or in the presence of dew, dew removal is a cultural practice implemented to manage dollar spot (Beard and Batten, 1982; Delvalle et al., 2011; Ellram et al., 2007; Koch, 2012). Other cultural methods include rolling using a standard lightweight turfgrass rolling machine (Giordano et al., 2012), fertility (Couch and Bloom, 1960; Golembiewski and Danneberger, 1998; Landschoot and McNitt, 1997; Markland et al., 1969; Watkins et al., 2001; Williams, et al., 1996), and removal of clippings (Williams et al., 1996). Irrigation programming has been investigated for its impact on dollar spot (Jiang et al., 1998; McDonald et al., 2006). The effect of leaf wetness on dollar spot has been studied (Walsh et al., 1999; Williams et al., 1996).

The effect of irrigation timing, frequency, application volume, and leaf wetness on various turfgrass diseases has been studied extensively (Couch and Bloom, 1960; Fidanza and Dernoeden, 1996; Gross et al., 1998; McDonald et al., 2006; Roberts et al., 2011; Watkins et al., 2001; Williams et al., 2001; Williams et al., 1996). Delvalle et al. (2011) demonstrated that daily dew removal reduced the severity of dollar spot compared with not removing dew and that dew removal extended the length of dollar spot control using fungicides. Williams et al. (1996) investigated the effects of dew removal in the morning (AM) at 0800 h and in the afternoon (PM) at 1300 h in

combination with fertility level and removal or return of clippings and found that AM dew removal resulted in significant reduction in dollar spot as compared to PM removal.

Many recommendations from university extension websites offer conflicting suggestions regarding the optimal time of day to irrigate turfgrass. Some of these publications suggest nighttime irrigation while others encourage avoidance of nighttime irrigation, citing an increased risk for disease potential. For the most part, according to these recommendations, the best time to water lawns falls into one of two categories: either water at nighttime or water in the morning. Some suggest the use of morning irrigation based on the idea that it will dilute guttation water which formed on plants overnight and can serve as a nutrient source for pathogens, and that nighttime irrigation will encourage disease development (Dernoeden, 2003; U of NH Cooperative Extension, 1994; Polomski and Shaughnessy, 1999; Fech, 2013; Voigt; Fresenburg, 2010; Pound and Street, 2013). Other extension articles suggest avoidance of morning irrigation, especially if the leaf wetness (LW) period is extended, and instead to irrigate at night because less evaporation occurs and winds are lower so water is presumed to be applied more efficiently (Smith, 2008; Toski and Skinner, 2012; Swift, 1996; Peacock and Bruneau, 2006). However, whether morning or nighttime irrigation was recommended, most of these publications typically state that the watering regime is most efficient when applied during less windy and cooler times of day so less evaporation takes place. Interestingly, there seems to be a general consensus that an important issue to consider when choosing when to water is that the LW period not be extended, which is said to encourage disease development.

Two of the most common, and often disputed, approaches are deep and infrequent or light and frequent irrigation scheduling. Deep and infrequent irrigation typically refers to watering which generally occurs two times weekly or less and usually involves applying enough water to penetrate into the soil approximately 3 cm beyond the root zone. Daily irrigation has been shown to reduce turfgrass quality (Jordan et al., 2003; Richie et al., 2002) and result in shallow rooting (Fu and Dernoeden, 2009; Jordan et al., 2003; Qian et al., 1997). However, benefits of daily irrigation have been reported which include improved turf quality (Fry and Huang, 2004, Fu and Dernoeden, 2009) and disease reduction (Jiang et al., 1998, McDonald et al., 2006, Melvin and Vargas, 1994).

Frequency of irrigation affects plants in several ways. Qian and Fry (1996) demonstrated that deep and infrequent irrigation resulted in more extensive rooting in Zoysiagrass (*Zoysia japonica* Steud.) as compared with light daily applications. Similarly, Fu and Dernoeden (2009) showed that creeping bentgrass under deep and infrequent irrigation led to the production of roots with a larger surface area, greater length, and were more numerous than turf irrigated on a light daily basis. Benefits of daily irrigation have been reported as well. JinMin and Dernoeden (2009) investigated watering frequency and found that watering creeping bentgrass on a light frequent basis resulted in very good color and quality throughout most of the trial period, while the deep infrequent program exhibited acceptable quality for one year only. In comparing light daily irrigation to infrequent irrigation, Melvin and Vargas (1994) observed less necrotic ring spot with daily irrigation as compared with infrequent irrigation programs.

McDonald et al. (2006) found deep and infrequent irrigation at 0600 h resulted in higher incidence of dollar spot than light and daily irrigation at 2100 h. Conversely, Miller (2012) demonstrated that deep and infrequently irrigated plots receiving 76 cm of irrigation annually resulted in lower dollar spot incidence compared to plots receiving 60-70 cm annually. In a greenhouse study, Couch and Bloom (1960) found that turf under higher levels of drought stress developed greater dollar spot incidence than turf watered to levels approaching field capacity. In a comparison of two irrigation regimes for their effect on dollar spot, Watkins et al. (2001) found that irrigation had no impact on dollar spot. Applying irrigation at 0500 h, Jiang et al. (1998) showed that plots receiving daily irrigated plots, while brown patch was suppressed with daily irrigation. Fidanza and Dernoeden (1996) discovered that morning irrigation (2200 h).

Researchers have investigated the effect ET has on irrigation of turfgrass and its impact on disease and quality. Evapotranspiration is the sum of amount of water lost from soil by evaporation and from plants by transpiration. Potential ET (PET) is the amount of water that would be lost by evaporation and transpiration if water was not limited in the system. PET can be used a predictor of irrigation requirements for crops. Numerous researchers have demonstrated that irrigation to 80% ET has resulted in acceptable turfgrass quality (DaCosta and Huang, 2006; Jiang et al., 1998; Richie et al., 2002; Roberts et al., 2011). Roberts et al. (2011) reported that plots irrigated to 80% ET typically exhibited the least amount of anthracnose and best turfgrass quality. Dacosta

and Huang (2006) showed that turfgrass quality was maintained at an acceptable level throughout the summer in New Jersey when turf was irrigated to 80% ET while in the fall of the year, 40% ET replacement maintained acceptable quality. Jiang et al. (1998) showed that plots receiving daily irrigation suppressed brown patch (*Rhizoctonia solani*) and had twice the number of dollar spot infection centers compared with those irrigated to 80% ET.

Creeping bentgrass is the most common cool season turfgrass used on golf courses (Lyman et al., 2007). Cultivars differ in susceptibility and may influence disease development. Turfgrass breeding efforts have led to improvements in disease resistance among many species of turfgrass (Belanger et al., 2004; Bonos, 2006; Bonos et al., 2006). Cultivars of creeping bentgrass have varying levels of susceptibility to diseases such as dollar spot (Casler et al., 2007; Cole et al., 1969; Settle et al., 2001) as well as differences in speed of recovery from diseases (Settle, et al., 2001; Vincelli et al., 1997). Settle et al. (2001) and Abernathy et al. (2001) identified 'L-93' as having higher dollar spot resistance than other cultivars in trials. Cole et al. (1969) revealed differences in dollar spot susceptibility and infection center diameter among creeping bentgrass cultivars.

The use of disease-resistant cultivars of creeping bentgrass may lead to a reduction in fungicide application rates or total number of fungicide applications made (Settle et al., 2001). Making curative rather than preventive fungicide applications for the control of dollar spot resulted in fewer fungicide applications made in more resistant creeping bentgrass cultivars by Settle et al. (2001). Vincelli et al. (1997) found that numerous cultivars of creeping bentgrass showed improved recovery from dollar spot

while many exhibited poor recovery. This indicates tremendous potential for reduced fungicide inputs when the use of less susceptible cultivars or those with faster recuperative potential are selected.

Many disease-resistant cultivars of creeping bentgrass with differing levels of resistance are commercially available; researchers have investigated the use of blended plantings. Abernathy et al. (2001) demonstrated that the use of resistant cultivars of creeping bentgrass in a blend suppressed dollar spot while the use of susceptible cultivars increased dollar spot. More recently, development of interspecific hybrids of dollar spot resistant creeping bentgrass and colonial bentgrass (*Agrostis capillaris* L.) created by Belanger et al. (2004) yielded hybrids with excellent dollar spot resistance in field tests. Based on 2010 National Turfgrass Evaluation Program (NTEP) progress report data, creeping bentgrass cultivars 'Declaration' and 'SRP-1WM' were considered to have relatively high resistance to dollar spot, while 'L-93' was comparatively more susceptible (NTEP, 2010). 'Crenshaw' is highly susceptible to dollar spot (NTEP, 2002).

Both irrigation program and cultivar selection can impact disease development; these factors were investigated in an integrated approach aimed at reducing dollar spot in creeping bentgrass. The objective of this research was to study the impact of three irrigation programs, specifically irrigating at 0500 h daily (daily morning), at 2200 h daily (daily nighttime), or at 2200 h twice weekly (infrequent nighttime), in combination with three creeping bentgrass cultivars ('Declaration', 'SRP-1WM', 'L-93') on DS incidence on fairway turf in East Lansing, Michigan.

Materials and Methods

Research was conducted at the Hancock Turfgrass Research Center on the campus of Michigan State University in East Lansing, MI from Jun 2011 through Sep 2013. Field plots were established in Oct 2010 on a renovated Colwood-Brookstone loam soil site. Nine 11 x 11 m irrigation plots were set up, each of which possessed a Toro TR50 sprinkler head (The Toro Company, Riverside, CA) located in plot corners. Within each irrigation plot, twelve plots measuring 2.7 x 3.7 m were established; four plots were seeded to one of three creeping bentgrass (*Agrostis stolonifera* L.) cultivars on 8 Oct 2010. Buffer strips surrounding irrigation plots were seeded to the dollar spot-susceptible cultivar 'Crenshaw'. Plots were mowed three times weekly at approximately 13 mm with clippings returned.

During the trial period in 2011, the study received a total of 146 kg N ha⁻¹ from either 18-9-18 or 24-2-12 granular fertilizer monthly. The herbicide Mec Amine-D (2,4-D, Mecoprop-p and Dicamba) (Loveland Products, Greeley, CO) was applied at 30 L ha⁻¹ on 21 Jun 2011 for the control of broadleaf weeds. Insects were controlled with carbaryl (Sevin; Bayer Environmental Science, Research Triangle Park, NC) on 21 Jul 2011, applied at 50 L ha⁻¹ for the control of black cutworms (*Agrotis ipsilon*).

Using a modified version of the Goodman and Burpee (1991) technique, the research site was inoculated with 210 kg ha⁻¹ of sand/cornmeal topdressing inoculum mixture on 17 Jun 2011 in order to encourage uniform disease development. A mixture of silica sand and cornmeal (Quaker Oats Co., Chicago, IL) (2:1, v/v) and 2.4% potato dextrose broth (Becton, Dickinson and Co, Sparks, MD) (5% v/v) was placed in 15 x 25

cm aluminum baking pans, covered with two layers of aluminum foil and placed into a second aluminum baking pan. The sand-cornmeal mixture was autoclaved at 121 C for 45 minutes. *S. homoeocarpa* on potato dextrose agar (PDA: 39 g PDA L⁻¹ water; Becton, Dickinson and Co, Sparks, MD) in 9 cm petri dishes was cut into 30-100 pieces and placed on the sterile sand-cornmeal. After incubation at 22 ± 2 C for 2 weeks, the media was chopped and kneaded by hand to break down clumps. The inoculum used was a mixture of equal proportions comprised of an isolate of *S. homoeocarpa* FT Bennett representing each of six different vegetative compatibility groups (VCGs) of the fungus which had previously been isolated from MI turfgrass affected by DS by Powell (1998). The blended sand-cornmeal inoculum was applied to field plots at a rate of 2.1 kg 100 m⁻² using a Gandy lawn spreader (Model 36H13, Gandy Co, Owatonna, MN). These VCGs were designated VCG A through VCG F.

The study was fertilized monthly in 2012 using 18-3-18 granular fertilizer totaling 122 kg N ha⁻¹ for the study duration. The herbicide amicarbazone (Xonerate; Arysta LifeScience North America LLC, Cary, NC) was applied to the study site at 360 ml ha⁻¹ in an effort to reduce *Poa annua* infiltration. To alleviate thatch build up in the turf, on 17 Aug 2012, plots were sand topdressed and the sand was worked into the turf using a greens mower (John Deere and Co., Moline, IL) fitted with Vibe V vibratory greens rollers (Turfline, Inc., Moscow Mills, MO). This was done to reduce scalping in the plots caused by mowing.

In 2012, no inoculations were planned. Instead, the fungal population sampled in 2011 was to be compared to that from 2013 to study impacts of treatment application on

the fungal population during that time period. However, due to lack of disease in the study site by early Sep 2012, the plots were re-inoculated on 13 Sep 2012 by spreading turfgrass clippings collected from an infested fairway at 40 kg ha⁻¹ using an Andersons SR2000 rotary fertilizer spreader (The Andersons, Inc., Maumee, OH). The clipping method was used in 2012 because it took considerably less time to prepare than the Goodman method employed in 2011.

The study received 120 kg N ha⁻¹ annually in 2013 using 18-3-18 granular fertilizer applied monthly during the trial period. On 29 Aug 2013, the plant growth regulator Trinexapac-ethyl (Primo Maxx; Syngenta Crop Protection, Inc, Greensboro, NC) was applied at 440 ml ha⁻¹.

On 6 Jun 2013, the study site was inoculated using sand/cornmeal topdressing inoculum at 160 kg ha⁻¹ as described for 2011. The topdressing mixture was infested with *S. homoeocarpa* VCG B only due to high virulence exhibited in laboratory and greenhouse screening by VCG B. Additionally, Deng et al (2002), Viji et al (2004), and DeVries (2006) found VCG B to be the most predominant in populations, while Powell and Vargas (2001) found VCG B to be among the most common VCG recovered from field isolations in Michigan.

Three irrigation programs were tested. Irrigation treatments were applied at 0500 h daily (daily morning), 2200 h daily (daily nighttime) or 2100 h twice weekly (infrequent nighttime) to three replicate plots. Toro TR50 sprinklers were located in plot corners and delivered 0.318 cm per 10 minutes to irrigation plots. Treatments applied on a daily basis were designed to replace approximately 80% PET. This rate was chosen based

on the results of researchers who demonstrated disease reduction based on 80% ET replacement (Jiang et al., 1998; Roberts et al., 2011). PET data were obtained from the Weather Enviro-weather Automated Station Network website (http://www.agweather.geo.msu.edu/mawn), formerly known as the Michigan Automated Weather Network (MAWN). The PET recorded from the website was used as a reference PET and was calculated based on the FAO Penman-Monteith equation (Allen et al., 1998.) Daily irrigation applications to replace 80% ET were adjusted twice weekly, similar to the technique used by Watkins et al. (2001). Average daily irrigation applications totaled 0.3, 0.4, and 0.2 cm in 2011, 2012, and 2013, respectively. Infrequent nighttime applications were made twice each week from Jun through mid-Sep, except as described below. Seasonal irrigation applications for daily irrigated plots were 31.2, 22.7, and 20.4 cm, and for infrequent irrigation, season totals were 29.9, 22.0, and 20.5 cm in 2011, 2012, and 2013, respectively. Approximately equivalent amounts of water were applied to all irrigation treatments weekly. Plots were not irrigated on 25-26 Jun and 27-28 Aug 2011, 10-15 Aug 2012, or 23-24 Aug 2013 due to heavy precipitation. Seasonal precipitation amounts during the trial period were 18.2, 11.4, and 16.7 cm for 13 Jun – 23 Sep 2011, 8 Jul – 11 Sep 2012, and 13 June – 7 Sep 2013, respectively.

The study site was renovated and plots were seeded in Oct 2010. Creeping bentgrass cultivars selected for this trial included both dollar spot resistant ('Declaration' and 'SRP-1WM') and DS susceptible ('L-93') representatives. These cultivars were chosen based on 2010 NTEP results (NTEP, 2010). Areas surrounding the irrigation

plots were seeded to 'Crenshaw', a highly susceptible cultivar, in order to promote the establishment and spread of dollar spot around the study site.

Plots were rated for dollar spot 10, 3, and 8 times in 2011, 2012, and 2013, respectively. Plots were visually rated for dollar spot incidence on a 0 to 100% scale, where 0 = no disease observed and 100 = entire plot area blighted. Area under the disease progress curve (AUDPC) values were calculated using the formula AUDPC = $\sum_{i=1}^{n-1} (\frac{y_i + y_{i+1}}{2})(t_{i+1} - t_i)$ where $i = 1, 2, 3... n - 1, y_i$ is the amount of disease (ie.

percent plot area blighted), and t_i is the *i*th rating (time in days) (Campbell and Madden, 1990). AUDPC is a measure of the average percent plot area blighted times the number of days between the ratings. Relative AUDPC (RAUDPC) was calculated for each season by dividing the AUDPC by the maximum potential AUDPC which is 100 times the trial duration in days (ie. 100*total trial days), and then multiplying by 100 to expand the scale. (Olanya and Campbell, 1990). This allows for simple comparisons across experiments since all data are converted to a 0-100 scale.

The trial consisted of a 3 x 3 factorial arrangement of treatments (three levels of irrigation regime and three levels of creeping bentgrass cultivar) in a randomized complete block design with split plots. Irrigation program was the whole plot treatment with three replications, and creeping bentgrass cultivar was the sub plot treatment with four replications within each whole plot. Disease data for 2011 and 2013 were square root transformed, but actual means for RAUDPC values are provided in the data tables. Data were subjected to analysis of variance (ANOVA) (least squares method) using JMP 10.0.0 (SAS Institute Inc., Cary, NC). Means were separated using Tukey's Honestly Significant Difference test (p=0.05).

Results

In 2011, dollar spot developed slowly through Jul and early Aug, and peaked in early Sep. Average dollar spot incidence data by irrigation program and by cultivar are provided in Figures 1 and 2. The main effects of irrigation regime, cultivar, and the interaction between the two were significant (Table 1).

Figure 1. 2011 Average dollar spot incidence among irrigation regimes. Values represent mean percent of plot area blighted by dollar spot averaged over all cultivars (n=36). Error bars represent the standard error of the mean (SEM).



Figure 2. 2011 Average dollar spot incidence among creeping bentgrass cultivars. Values represent mean percent of plot area blighted by dollar spot averaged over all irrigation treatments (n=36). Error bars represent the standard error of the mean (SEM).



The interaction of cultivar 'L-93' with daily nighttime irrigation resulted in significantly less dollar spot than with infrequent nighttime irrigation even though approximately equivalent volumes of water were applied weekly (Table 1). Likewise, the interaction of 'Declaration' with daily nighttime irrigation resulted in significantly less dollar spot than 'Declaration' with infrequent nighttime irrigation. 'SRP-1WM' with daily nighttime irrigation resulted in significantly less dollar spot than 'Declaration' with infrequent nighttime irrigation. 'SRP-1WM' with daily nighttime irrigation resulted in significantly less dollar spot than 'SRP-1WM' with infrequent nighttime irrigation (Figure 3). Daily nighttime irrigation programs exhibited significantly less dollar spot in 'L-93' and 'Declaration' than the daily morning irrigation programs for 'L-93' and 'Declaration', but those same irrigation treatments for 'SRP-1WM' were not statistically different (Table 1.) Plots receiving daily morning irrigation were not significantly different than those irrigated infrequently during the night for all cultivars (Table 1).

The RAUDPC was calculated for the period from 13 Jul through 23 Sep 2011, a total of 71 days, and ranged from 1.02 to 40.63. RAUDPC values indicated that daily nighttime irrigation programs had significantly lower incidence of dollar spot than daily morning irrigation or infrequent nighttime irrigation programs. Over the duration of the season, 'SRP-1WM' exhibited the lowest RAUDPC dollar spot value among all cultivars, while 'Declaration' developed significantly more dollar spot than 'SRP-1WM' but less than 'L-93'. 'L-93' was the most susceptible cultivar, resulting in significantly higher RAUDPC dollar spot levels among all cultivars over all irrigation programs.

	RAUDPC ((AUDPC/Maximum Potential AUDPC) x 100) ^a				
	'L-9	93'	'Declai	ration'	'SRP-1WM'
Infrequent nighttime	40.63	a ^b	21.13	С	8.01 de
Daily morning	37.07	а	17.81	С	2.85 ef
Daily nighttime	28.43	b	9.87	d	1.02 f
Irrigation Program					
Infrequent nighttime	23.26	а			
Daily morning	19.24	b			
Daily nighttime	13.11	С			
Cultivar					
'L-93'	35.38	а			
'Declaration'	16.27	b			
'SRP-1WM'	3.96	С			
Analysis of Variance					
Irrigation (I)	<.0001				
Cultivar (C)	<.0001				
IxC	0.0428				

Table 1. The effect of irrigation regime and creeping bentgrass cultivar on dollar spot in 2011.

^a Data were analyzed using a square root transformation, but actual means are shown.

^b For main effects of Irrigation Program and Cultivar, means in the same column followed by the same letter are not significantly different while interactions are compared among all treatments at p=0.05 according to Tukey's HSD.

Figure 3. 2011 Relative Area Under the Disease Progress Curve (RAUDPC) for irrigation program and creeping bentgrass cultivar. The effect of irrigation scheduling program on dollar spot in three creeping bentgrass cultivars measured as RAUDPC from 13 Jul through 23 Sep 2011, a total of 71 days. RAUDPC values were the calculated means (n=12) of each irrigation x cultivar interaction and were calculated as described in the text. Bars sharing common letters are not significantly different at p=0.05 for all interactions (Tukey's HSD).



Dollar spot pressure was extremely low for the 2012 season. On average, less than 1% plot area was blighted with dollar spot (based only on three rating dates, data not shown). As a result, RAUDPC was extremely low (0.00 to 0.81). Although statistically significant, dollar spot epidemics in 2012 did not reach levels to which informative or conclusive inferences could be made, and thus will not be discussed or presented.

Disease pressure developed steadily through Jul and early Aug in 2013 and continued to build into early Sep (Figures 4 and 5). No significant interaction between irrigation program and cultivar was found. When applying approximately the same weekly volume of water, daily irrigated plots, whether morning or nighttime, developed significantly less dollar spot than those irrigated infrequently during the night for all cultivars in 2013 (Table 2). Dollar spot incidence was lower under a daily nighttime irrigation regime as compared with daily morning watering (Table 2). Similar to results from 2011, 'L-93' was the most susceptible cultivar, exhibiting the highest level of dollar spot. 'SRP-1WM' was consistently the most resistant cultivar in the study (Table 2).

Figure 4. 2013 Average dollar spot incidence among irrigation regimes. Values represent mean percent of plot area blighted by dollar spot averaged over all cultivars (n=36). Error bars represent the standard error of the mean (SEM).



Figure 5. 2013 Average dollar spot incidence among creeping bentgrass cultivars. Values represent mean percent of plot area blighted by dollar spot averaged over all irrigation treatments (n=36). Error bars represent the standard error of the mean (SEM).



	RAUDPC ((AUDPC/Maximum			
	Potential AUDPC) x 100) ^a			
Irrigation				
Infrequent nighttime	29.09	a ^b		
Daily morning	21.65	b		
Daily nighttime	18.04	С		
Cultivar				
'L-93'	38.58	а		
'Declaration'	16.66	b		
'SRP-1WM'	13.53	С		
ANOVA				
Irrigation (I)	<.0001			
Cultivar (C)	<.0001			
IxC	>0.05			

 Table 2. The effect of irrigation regime and creeping bentgrass cultivar on dollar spot in 2013.

^a Data were analyzed using a square root transformation, but actual means are shown.

^b For main effects of Irrigation Program and Cultivar, means in the same column followed by the same letter are not significantly different while interactions are compared among all treatments at p=0.05 according to Tukey's HSD.

The RAUDPC values were calculated for the season from 1 Jul through 18 Sep 2013 and ranged from 13.53 to 29.02 (Table 2). When comparing the effect of irrigation regime on each cultivar independently, both of the daily irrigation programs resulted in significantly less dollar spot incidence than those irrigated infrequently at night for 'L-93' and 'SRP-1WM' (Figure 6). For 'Declaration', the daily nighttime watering program had significantly less dollar spot than all other irrigation treatments, although plots irrigated daily in the morning had significantly less dollar spot than all other spot than those on the infrequent nighttime program (Figure 6). Within each cultivar, infrequent nighttime irrigation resulted in significantly higher dollar spot levels than either of the daily irrigation programs (Figure 6).

Figure 6. 2013 Relative Area Under the Disease Progress Curve (RAUDPC) for irrigation program and creeping bentgrass cultivar. The effect of irrigation scheduling program on dollar spot in three creeping bentgrass cultivars measured as RAUDPC from 1 Jul through 18 Sep 2013, a total of 79 days. RAUDPC values were the calculated means (n=12) of each irrigation x cultivar combination and were calculated as described in the text. Since there was no significant interaction between irrigation regime and cultivar, comparisons among irrigation programs were made within each cultivar. Bars sharing common letters are not significantly different at p=0.05 for all interactions (Tukey's HSD).



Discussion

Application of equivalent weekly volumes of irrigation to a fairway in MI on a daily basis, as compared to an infrequent basis, resulted in a significant reduction in dollar spot incidence. This conclusion supports work conducted by McDonald et al. (2006) that showed that plots receiving daily irrigation had lower incidence of dollar spot when compared to infrequent irrigation. Although the frequency of application was either daily or infrequent (twice weekly) in each of the studies, the time of day for the applications and the total weekly amount of water applied differed in that study. McDonald et al. (2006) compared light and frequent (daily) irrigation applications made at 2100 h with infrequent applications made at 0600 h; there was no comparison made between different irrigation frequencies made at the same time of day. Additionally, efforts to apply the same amount of water to frequent and infrequent irrigation plots was attempted for the first year of the study, after which irrigation amounts applied to each treatment varied due to high amounts of natural precipitation which affected application parameters for the study (McDonald et al., 2006).

In evaluating the effects of irrigation frequency on dollar spot severity in the field, the findings of Jiang et al. (1998) and Watkins et al (2001) were not supported. Jiang et al. (1998) reported that severity of dollar spot was enhanced by daily irrigation compared with irrigation 3 times weekly at a level to return 80% evapotranspiration (ET); however, irrigation volumes were 200% higher in the plots that were irrigated daily compared to those irrigated 3 times weekly. This large difference in water application may have influenced the outcome of the study. Efforts were made in the current study to maintain equivalent water applications to all plots on a weekly basis and the outcomes

suggested that incidence of dollar spot was impacted by the time of day or frequency of irrigation applications rather than volume of water applied.

The results of this study demonstrated that nighttime irrigation on a daily basis resulted in a reduction in dollar spot. Benefits reported for nighttime irrigation include a reduction in loss of water from evaporation than daytime irrigation, providing more efficient use of irrigation water (Smith, 2008). Nighttime irrigation may also dilute and displace dew on turf plants. The extension of the duration of the leaf wetness period in turf has been shown to increase disease incidence (Gross et al., 1998; Uddin et al., 2003; Vargas, et al., 1993). However, if dew is already present as was the case in this study, the duration of the leaf wetness period would not be extended by watering at 2200 h as would potentially occur by watering at 1700 h, for example. The duration of the leaf wetness period would be extended in the case of late day or evening irrigation which could lead to increased disease potential. In Kentucky, Williams et al. (1998) showed that leaf wetness duration did not appear to be reduced by any significant factor when dew was removed prior to 0400 h. In that trial, dew was already present on the turf at 2200 h.

The development of improved cultivars of turfgrass with greater levels of disease resistance has impacted disease epidemics. Research focusing on development of dollar spot on resistant creeping bentgrass cultivars has increased the number made commercially available. Results indicate that since 'L-93' is more susceptible than either 'Declaration' or 'SRP-1WM', choosing a dollar spot resistant cultivar may allow turfgrass managers to reduce fungicide inputs for dollar spot management. By combining this potential with careful cultural management practices, it is anticipated that even greater

reductions in pesticide use could be attained. The importance of cultivar selection should not be underestimated when designing integrated disease management programs.

By utilizing less susceptible creeping bentgrass cultivars and combining this effort with an irrigation program designed to reduce disease incidence, less dollar spot was achieved in this study. The direct comparison of daily versus infrequent irrigation, with time of day and weekly volume applied held equivalent, indicated that daily irrigation resulted in less dollar spot than infrequent irrigation. Although infrequent nighttime and daily morning watering programs were similar in 2011 and significantly different in 2013, with daily morning irrigation resulting in less dollar spot, this comparison is not entirely justified since time of day of application was different. A better comparison would have been to include an infrequent morning program to compare with daily morning programs. However, due to space limitations, this was not possible in the current trial but would be interesting to investigate in future studies. Irrigating daily as compared with infrequently at 2200 h using the same weekly volume of water resulted in less dollar spot on fairway turf in MI. Results were not conclusive when comparing morning and nighttime daily irrigation regimes since differences occurred for some cultivars but not others between the 2011 and 2013 seasons. This could be due to variation in evapotranspiration rates among cultivars.

It is possible that watering at 2200 h diluted guttation fluids and delayed fungal growth as compared with irrigating at 0500 h. Daily irrigation may prevent drought stress in turfgrass while plants under higher drought stress are more susceptible to disease. Additionally, plots receiving daily irrigation may experience an elevation in bacterial

populations compared with those receiving infrequent irrigation. Those plots being watered daily may avoid a dry down period experienced in plots receiving less frequent irrigation programming. Moisture is highly influential with regards to soil bacterial populations, some of which can be inhibitory to pathogens.

Due to reduced dollar spot incidence observed in this study, daily nighttime watering would be the preferred method of irrigation where dollar spot is the major disease. Additionally, the amount of dollar spot may be reduced with the use of resistant cultivars. Combining host resistance with daily nighttime irrigation could possibly reduce the use of fungicides for dollar spot by reducing disease incidence, thereby having a positive impact on the environment and budget for crop protection.
APPENDIX

APPENDIX

	Ju	June		uly	Aug	gust	September		
	Air Max	Air Min	Air Max	Air Min	Air Max	Air Min	Air Max	Air Min	
Day	(°F)*	(°F)*	(°F)	(°F)	(°F)	(°F)	(°F)	(°F)	
1	74.8	61.4	85.6	58.4	88.8	71.6	89.1	64.1	
2	71.0	50.5	91.6	68.9	85.7	68.3	90.5	71.1	
3	75.6	51.6	86.7	66.1	84.0	70.2	88.7	68.5	
4	89.4	60.7	85.4	62.7	80.8	64.9	73.4	58.9	
5	85.4	56.8	87.6	57.7	84.5	61.9	59.5	48.1	
6	84.7	55.6	86.1	67.6	80.8	69.9	66.6	46.2	
7	92.9	73.8	79.6	56.5	81.1	66.3	67.2	48.9	
8	91.4	74.9	86.1	56.8	81.2		64.4	55.6	
9	82.4	57.4	89.4	60.2	79.6	63.2	76.7	59.1	
10	60.0	52.9	89.0	66.2	73.9	60.4	78.8	60.1	
11	75.2	58.1	80.1	66.8	77.8	53.3	77.6	57.5	
12	66.2	50.3	86.1	66.2	78.7	55.2	81.8	58.9	
13	78.4	52.2	78.1	59.4	79.5	60.6	76.4	54.2	
14	76.5	50.1	80.0	51.5	72.9	58.4	65.4	43.8	
15	67.3	47.5	84.5	61.8	79.2	56.5	59.8	38.9	
16	73.0	57.7	90.9	57.4	83.3	53.5	58.9	36.3	
17	77.8	53.2	91.2	64.8	80.6	55.4	64.8	45.9	
18	82.6	56.0	90.4	74.6	82.8	62.7	69.3	43.8	
19	80.2	56.6	93.0	72.6	83.4	53.3	66.7	55.9	
20	76.0	59.8	92.9	70.3	80.7	59.8	71.0	46.5	
21	86.8	66.8	93.9	72.0	76.7	56.5	74.7	51.9	
22	79.9	63.9	82.0	65.7	76.2	49.3	67.4	49.8	
23	70.1	62.1	86.8	67.7	80.3	53.2	63.9	50.9	
24	64.5	58.3	86.3	67.2	87.8	65.5	64.8	43.9	
25	77.7	57.4	86.9	71.4	76.9	58.9	70.1	49.5	
26	81.1	52.2	82.5	65.0	79.5	51.5	69.3	49.1	
27	78.3	58.1	77.4	56.5	81.9	55.9	65.7	43.8	
28	70.9	61.3	86.1	69.2	75.1	54.8	61.7	52.0	
29	78.7	53.3	87.2	70.4	76.4	47.9	62.4	49.3	
30	83.0	51.4	88.2	62.4	77.2	53.7	52.3	39.9	
			88.0	64.2	79.9	61.2			

 Table 3. 2011 Daily average maximum/minimum air temperatures.

*Air temperature taken at 1.5 m from ground. Source: Enviro-weather (http://www.agweather.geo.msu.edu/mawn/station.asp?id=htc)

	Ju	ne	Ju	lly	Aug	gust	Sept	ember
	Air Max	Air Min						
Day	(°F)*	(°F)*	(°F)	(°F)	(°F)	(°F)	(°F)	(°F)
1	53.0	46.1	90.8	61.0	83.8	60.6	80.8	61.2
2	70.2	46.1	94.7	61.0	89.0	61.7	83.2	64.2
3	75.7	56.8	92.4	70.1	94.2	66.0	89.9	60.2
4	71.2	52.2	96.9	72.4	89.4	68.1	83.9	66.1
5	68.5	49.1	95.7	68.4	81.8	66.1	79.0	63.9
6	77.5	43.8	100.9	68.9	83.1	50.7	83.7	63.7
7	78.7	51.1	92.3	73.2	86.4	54.5	76.4	57.8
8	80.5	49.3	83.2	63.9	83.7	65.1	68.0	52.9
9	84.5	62.7	86.2	57.5	71.4	58.6	71.2	47.9
10	88.2	58.7	82.6	61.1	62.5	57.7	74.0	41.8
11	80.3	64.8	85.2	54.7	72.2	57.2	79.1	51.0
12	74.5	57.7	88.0	53.9	76.5	55.9	82.4	55.4
13	72.7	47.5	90.4	59.4	70.9	60.0	78.4	55.7
14	79.0	47.8	90.3	63.8	78.0	60.6	70.4	51.5
15	85.7	53.3	88.4	68.0	82.6	56.9	73.6	41.6
16	86.9	62.6	92.6	65.9	75.9	60.9	76.3	47.9
17	80.0	59.6	95.9	75.2	73.4	57.1	75.7	50.7
18	79.6	57.6	84.7	68.0	75.1	44.9	64.4	43.5
19	91.5	73.4	74.8	65.9	77.6	51.0	65.0	38.8
20	90.1	71.3	80.5	60.0	75.6	49.2	68.3	47.7
21	84.8	62.9	85.1	53.9	78.1	49.2	67.1	43.4
22	80.5	58.2	89.2	65.1	80.3	49.1	58.7	44.2
23	82.7	52.8	93.4	71.7	86.9	50.4	55.8	40.6
24	84.2	64.7	83.2	65.6	88.2	58.6	61.9	37.5
25	73.8	54.7	88.4	55.8	89.7	58.6	67.7	49.5
26	80.7	46.6	84.5	69.3	88.7	63.8	71.3	49.6
27	86.7	52.7	79.3	65.7	84.4	67.9	65.0	47.2
28	94.5	66.2	80.9	59.9	78.9	58.6	69.2	45.7
29	87.0	64.5	85.5	53.8	81.2	50.7	71.7	40.3
30	87.5	65.0	87.6	59.6	84.2	51.6	64.4	44.8
			85.4	62.7	90.9	68.1		

 Table 4. 2012 Daily average maximum/minimum air temperatures.

*Air temperature taken at 1.5 m from ground. Source: Enviro-weather (http://www.agweather.geo.msu.edu/mawn/station.asp?id=htc)

	Ju	ine	Ju	ıly	Aug	gust	Septe	ember
	Air Max	Air Min						
Day	(°F)*	(°F)*	(°F)	(°F)	(°F)	(°F)	(°F)	(°F)
1	76.3	63.3	72.4	55.8	75.7	60.7	81.9	65.3
2	67.4	50.1	72.0	55.3	70.7	54.3	71.5	58.4
3	67.6	40.0	80.9	59.5	76.7	53.0	73.6	56.6
4	71.1	48.1	78.0	59.5	73.6	56.2	78.2	51.5
5	68.7	50.4	84.2	62.9	69.8	50.2	70.4	50.8
6	68.0	54.7	83.3	65.7	78.1	61.3	75.1	46.0
7	67.2	49.8	81.4	67.3	81.4	63.0	79.0	57.7
8	73.4	46.2	80.2	67.4	77.2	59.0	72.6	57.1
9	76.3	57.1	86.3	65.9	78.7	53.0	81.9	54.4
10	73.4	59.9	84.4	66.8	79.5	58.8	90.2	70.2
11	79.4	62.1	79.6	57.8	76.9	57.5	86.3	69.3
12	73.6	59.1	80.2	53.1	71.4	61.8	77.0	55.7
13	78.4	60.3	82.5	56.5	67.8	53.2	59.8	40.8
14	76.9	55.2	88.2	61.6	70.3	48.6	66.9	38.1
15	75.9	54.6	92.1	67.7	72.1	44.9	59.3	46.0
16	78.6	62.6	89.8	72.2	78.8	48.1	61.9	44.9
17	83.8	58.0	90.8	72.2	78.8	48.6	67.1	33.4
18	70.6	52.9	90.6	71.5	81.4	51.6	75.2	44.3
19	75.3	45.9	91.1	74.2	80.6	53.1	77.8	57.5
20	81.2	51.6	84.4	69.2	83.4	58.7	73.2	65.5
21	84.8	59.6	82.6	61.7	84.7	63.5	66.1	53.8
22	86.4	67.2	80.3	65.6	78.9	64.2	54.6	39.3
23	87.0	68.7	79.5	58.4	80.6	58.9	62.1	35.0
24	85.5	66.2	72.7	51.6	81.4	52.7	67.4	35.8
25	81.1	65.6	77.2	49.3	83.8	56.6	73.6	39.3
26	81.2	62.5	77.4	57.5	83.5	68.0	76.0	38.3
27	84.3	65.1	74.1	55.1	86.4	69.6	76.5	40.7
28	78.3	62.9	64.4	49.7	82.2	66.7	76.6	45.4
29	73.1	62.6	68.1	55.7	88.4	61.4	68.4	47.7
30	77.4	60.2	79.3	46.9	85.1	66.5	69.8	44.9
			67.2	63.1	78.3	65.9		

Table 5. 2013 Daily average maximum/minimum air temperatures.

*Air temperature taken at 1.5 m from ground. Source: Enviro-weather (http://www.agweather.geo.msu.edu/mawn/station.asp?id=htc)

	June			July				August		September		
Dav	Tot. Ppt.ª	Avg. Rel. Hum. [⋼]	Est. PET°	Tot. Ppt.	Avg. Rel. Hum.	Est. PET	Tot. Ppt.	Avg. Rel. Hum.	Est. PET	Tot. Ppt.	Avg. Rel. Hum.	Est. PET
1		44.7	0.27		64.8	0.19		64.9	0.21		69.9	0.17
2		49.7	0.20		67.9	0.23	0.06	72.1	0.11	0.01	65.9	0.19
3		61.5	0.19		60.2	0.22	0.77	77.0	0.13		73.1	0.16
4		60.2	0.22		49.5	0.24		79.5	0.09	0.11	74.5	0.11
5		54.7	0.22		58.2	0.24		72.3	0.17		70.5	0.08
6		57.7	0.21		53.4	0.23	0.09	83.3	0.07		70.0	0.11
7		58.5	0.24		59.3	0.17		79.4	0.13	0.06	79.5	0.08
8		52.1	0.27	-	57.3	0.22	0.02	75.4	0.12	-	84.1	0.04
9	0.01	69.0	0.13		55.3	0.21	0.28	74.6	0.2	0.33	81.4	0.08
10	0.44	83.8	0.05		57.6	0.21		63.1	0.2	0.04	79.3	0.11
11		80.6	0.11	0.34	78.4	0.09		60.8	0.2		79.2	0.11
12		68.2	0.11		63.2	0.23		68.9	0.2		73.4	0.15
13		47.7	0.22		57.9	0.21	0.31	79.4	0.1		65.2	0.13
14		53.9	0.22		59.6	0.19	0.17	87.7	0.1	0.16	78.5	0.06
15	0.05	77.6	0.10		61.0	0.18		69.1	0.2		62.5	0.11
16	0.51	75.3	0.14		57.1	0.22		66.6	0.2		71.4	0.07
17		74.4	0.12		60.7	0.23		70.9	0.2		66.7	0.10
18		67.3	0.21	0.20	71.6	0.16	0.01	67.5	0.2		72.3	0.12
19		62.2	0.20		64.9	0.20		65.4	0.2	0.73	87.9	0.04
20		77.3	0.11		68.3	0.20	0.49	80.8	0.1	0.01	78.2	0.11
21	0.24	78.9	0.16		59.5	0.26	0.02	68.5	0.2		74.3	0.11
22	0.26	78.1	0.14	0.03	75.7	0.09	-	63.9	0.2		73.7	0.09
23	0.02	80.4	0.08	0.02	76.2	0.14	0.45	70.3	0.2		81.1	0.06
24	0.05	82.7	0.07		74.9	0.15	0.41	73.9	0.2		78.6	0.09
25		62.3	0.21		62.9	0.22		68.5	0.1	0.02	84.6	0.05
26		60.3	0.21		53.2	0.23		73.9	0.2	0.61	82.0	0.06
27		72.7	0.12	1.28	79.6	0.09		69.3	0.2	0.09	79.1	0.06
28		67.0	0.17	1.62	80.4	0.14		63.1	0.2		86.3	0.04
29		60.5	0.22	1.61	72.4	0.19		66.6	0.2	0.35	88.5	0.04
30		65.7	0.18		60.7	0.22		68.9	0.1	0.13	81.7	0.05
31					65.3	0.23		75.5	0.1			0.11

Table 6. 2011 Daily total precipitation, average relative humidity, and estimated potential evapotranspiration (PET).

^a Total precipitation (inches).
 ^b Average relative humidity (percent).
 ^c Estimated PET (inches per day).
 Source: Enviro-weather (http://www.agweather.geo.msu.edu/mawn/station.asp?id=htc)

Avg. Ppt. Avg. Rel. Hum. Avg. PET Avg. PET Avg. Rel. PET Avg. PET Avg. Rel. PET Avg. PET 1 0.58 90.3 0.03 56.9 0.21 59.9 0.20 62.5 0.16 62.9 0.13 4 60.3 0.17 59.9 0.23 0.11 66.6 0.19 0.64 73.2 0.09 5 66.5 0.15 0.03 67.1 0.18 54.0 0.21 63.4		June			July			August			September		
Tot.Rel.Est.T		_	Avg.			Avg.			Avg.			Avg.	
Day Ppt. Hum. Ppt. Sup.	Dav	Tot.	Rel.	Est.	Tot.	Rel.	Est.	Tot.	Rel.	Est.	Tot.	Rel.	Est.
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Day	- Ρρι.			Ρρι.			Ρρι.			Ρρι.		0 10
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	1	0.58	90.3	0.03		56.9	0.21		59.9	0.20		59.5	0.12
3 53.3 0.23 60.5 0.21 58.7 0.21 0.05 65.3 0.16 4 60.3 0.17 59.9 0.23 0.11 66.6 0.19 0.64 73.2 0.09 5 66.5 0.15 0.03 67.1 0.18 60.4 0.23 79.1 0.10 6 0.01 59.6 0.21 61.2 0.23 54.0 0.21 63.4 0.14 7 56.4 0.21 61.2 0.23 57.0 0.20 0.28 68.3 0.07 8 51.0 0.22 54.3 0.22 52.0 0.19 0.06 69.0 0.12 9 44.7 0.25 57.1 0.18 0.43 83.9 0.03 $$ 63.1 0.13	2		63.8	0.17		56.0	0.20		62.5	0.16		62.9	0.13
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	3		53.3	0.23		60.5	0.21		58.7	0.21	0.05	65.3	0.15
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	4		60.3	0.17		59.9	0.23	0.11	66.6	0.19	0.64	73.2	0.09
6 0.01 59.6 0.21 $$ 61.2 0.23 $$ 54.0 0.21 $$ 63.4 0.14 7 $$ 56.4 0.21 $$ 63.2 0.23 $$ 57.0 0.20 0.28 68.3 0.07 8 $$ 51.0 0.22 $$ 54.3 0.22 $$ 52.0 0.19 0.06 69.0 0.12 9 $$ 44.7 0.25 $$ 57.1 0.18 0.43 83.9 0.03 $$ 61.8 0.13 10 $$ 51.6 0.24 $$ 50.8 0.21 0.70 90.4 0.04 $$ 63.1 0.13 11 0.02 66.5 0.15 $$ 49.7 0.21 0.38 82.0 0.07 $$ 57.5 0.16 12 0.02 57.1 0.23 $$ 49.7 0.21 0.38 82.0 0.07 $$ 57.5 0.16 13 $$ 52.5 0.19 $$ 56.9 0.21 0.03 80.3 0.05 0.20 67.5 0.10 14 $$ 50.6 0.22 0.20 60.8 0.22 $$ 68.7 0.15 $$ 59.9 0.13 15 $$ 52.3 0.25 $$ 66.4 0.22 $$ 68.7 0.15 $$ 59.9 0.13 16 $$ 60.4 0.21 $$ 58.6 0.23 <	5		66.5	0.15	0.03	67.1	0.18		60.4	0.23		79.1	0.10
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	6	0.01	59.6	0.21		61.2	0.23		54.0	0.21		63.4	0.14
851.0 0.22 54.3 0.22 52.0 0.19 0.06 69.0 0.12 944.7 0.25 57.1 0.18 0.43 83.9 0.03 61.8 0.13 1051.6 0.24 50.8 0.21 0.70 90.4 0.04 63.1 0.13 11 0.02 66.5 0.15 49.7 0.21 0.38 82.0 0.07 57.5 0.16 12 0.02 57.1 0.23 49.7 0.21 0.38 82.0 0.07 57.5 0.16 13 52.5 0.19 56.9 0.21 0.03 80.3 0.05 0.20 67.5 0.10 14 50.6 0.22 0.20 60.8 0.22 0.5 73.3 0.15 0.36 61.8 0.13 15 52.3 0.25 66.4 0.22 68.7 0.15 59.9 0.13 16 60.4 0.21 58.6 0.23 0.06 76.0 0.10 59.9 0.11 17 63.3 0.15 59.2 0.29 67.9 0.15 0.01 59.9 0.11	7		56.4	0.21		63.2	0.23		57.0	0.20	0.28	68.3	0.07
9 44.7 0.25 57.1 0.18 0.43 83.9 0.03 61.8 0.13 10 51.6 0.24 50.8 0.21 0.70 90.4 0.04 63.1 0.13 11 0.02 66.5 0.15 49.7 0.21 0.38 82.0 0.07 57.5 0.16 12 0.02 57.1 0.23 49.7 0.21 0.38 82.0 0.07 57.5 0.16 13 52.5 0.19 56.9 0.21 0.03 80.3 0.05 0.20 67.5 0.10 14 50.6 0.22 0.20 60.8 0.22 0.05 73.3 0.15 0.36 61.8 0.13 15 52.3 0.25 66.4 0.22 68.7 0.15 59.9 0.13 16 60.4 0.21 58.6 <	8		51.0	0.22		54.3	0.22		52.0	0.19	0.06	69.0	0.12
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	9		44.7	0.25		57.1	0.18	0.43	83.9	0.03		61.8	0.13
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	10		51.6	0.24		50.8	0.21	0.70	90.4	0.04		63.1	0.13
12 0.02 57.1 0.23 49.9 0.22 66.7 0.16 55.2 0.16 13 52.5 0.19 56.9 0.21 0.03 80.3 0.05 0.20 67.5 0.16 14 50.6 0.22 0.20 60.8 0.22 0.05 73.3 0.15 0.36 61.8 0.13 15 52.3 0.25 66.4 0.22 68.7 0.15 59.9 0.13 16 60.4 0.21 58.6 0.23 0.06 76.0 0.10 56.0 0.13 17 63.3 0.15 50.2 0.29 67.9 0.15 0.01 59.9 0.14	11	0.02	66.5	0.15		49.7	0.21	0.38	82.0	0.07		57.5	0.16
13 52.5 0.19 56.9 0.21 0.03 80.3 0.05 0.20 67.5 0.10 14 50.6 0.22 0.20 60.8 0.22 0.05 73.3 0.15 0.36 61.8 0.13 15 52.3 0.25 66.4 0.22 68.7 0.15 59.9 0.13 16 60.4 0.21 58.6 0.23 0.06 76.0 0.10 56.0 0.13 17 63.3 0.15 50.2 0.29 67.9 0.15 0.01 59.9 0.11	12	0.02	57.1	0.23		49.9	0.22		66.7	0.16		55.2	0.16
14 50.6 0.22 0.20 60.8 0.22 0.05 73.3 0.15 0.36 61.8 0.13 15 52.3 0.25 66.4 0.22 68.7 0.15 59.9 0.13 16 60.4 0.21 58.6 0.23 0.06 76.0 0.10 56.0 0.13 17 63.3 0.15 50.2 0.29 67.9 0.15 0.01 59.9 0.11	13		52.5	0.19		56.9	0.21	0.03	80.3	0.05	0.20	67.5	0.10
15 52.3 0.25 66.4 0.22 68.7 0.15 59.9 0.13 16 60.4 0.21 58.6 0.23 0.06 76.0 0.10 56.0 0.13 17 63.3 0.15 50.2 0.29 67.9 0.15 0.01 59.9 0.11	14		50.6	0.22	0.20	60.8	0.22	0.05	73.3	0.15	0.36	61.8	0.13
16 60.4 0.21 58.6 0.23 0.06 76.0 0.10 56.0 0.13 17 63.3 0.15 50.2 0.29 67.9 0.15 0.01 59.9 0.11	15		52.3	0.25		66.4	0.22		68.7	0.15		59.9	0.13
	16		60.4	0.21		58.6	0.23	0.06	76.0	0.10		56.0	0.13
	17		63.3	0.15		50.2	0.29		67.9	0.15	0.01	59.9	0.11
18 0.37 72.7 0.14 0.40 74.1 0.14 61.4 0.16 0.19 63.6 0.09	18	0.37	72.7	0.14	0.40	74.1	0.14		61.4	0.16	0.19	63.6	0.09
19 52.9 0.26 0.26 84.7 0.08 65.5 0.16 0.01 60.0 0.13	19		52.9	0.26	0.26	84.7	0.08		65.5	0.16	0.01	60.0	0.13
20 51.5 0.26 0.01 64.9 0.19 63.4 0.15 0.06 57.6 0.13	20		51.5	0.26	0.01	64.9	0.19		63.4	0.15	0.06	57.6	0.13
21 0.05 64.2 0.18 62.1 0.20 60.4 0.17 0.16 60.5 0.09	21	0.05	64.2	0.18		62.1	0.20		60.4	0.17	0.16	60.5	0.09
22 63.1 0.19 62.5 0.19 61.4 0.17 0.06 69.4 0.07	22		63.1	0.19		62.5	0.19		61.4	0.17	0.06	69.4	0.07
23 57.8 0.20 0.04 57.6 0.25 60.2 0.17 0.06 67.0 0.08	23		57.8	0.20	0.04	57.6	0.25		60.2	0.17	0.06	67.0	0.08
24 56.7 0.19 55.7 0.22 54.4 0.18 0.01 60.1 0.12	24		56.7	0.19		55.7	0.22		54.4	0.18	0.01	60.1	0.12
25 53.9 0.21 0.02 59.9 0.21 56.2 0.19 0.02 65.3 0.10	25		53.9	0.21	0.02	59.9	0.21		56.2	0.19	0.02	65.3	0.10
26 52.1 0.21 0.19 76.2 0.11 0.12 61.4 0.18 0.01 63.7 0.09	26		52.1	0.21	0.19	76.2	0.11	0.12	61.4	0.18	0.01	63.7	0.09
27 50.5 0.24 80.0 0.12 0.20 65.9 0.14 63.2 0.09	27		50.5	0.24		80.0	0.12	0.20	65.9	0.14		63.2	0.09
			50.2	0.25		64.4	0.20		60.8	0.16		61.2	0.10
	29		54.6	0.20		58.4	0.19		63.1	0.15		59.0	0 11
	30		56 1	0.23		56.7	0.21		61.6	0 17		67.2	0.09
	31		00.1	0.20	0.31	66.5	0.10		48.6	0.21		01.2	0.00

Table 7. 2012 Daily total precipitation, average relative humidity, and estimated potential evapotranspiration (PET).

^a Total precipitation (inches).
 ^b Average relative humidity (percent).
 ^c Estimated PET (inches per day).
 Source: Enviro-weather (http://www.agweather.geo.msu.edu/mawn/station.asp?id=htc)

	June			July			August			September		
		Avg.			Avg.			Avg.			Avg.	
Dov	Tot.	Rel.	Est.	Tot.	Rel.	Est.	Tot.	Rel.	Est.	Tot.	Rel.	Est.
Day	- рі. 0 ре			- μι.	F0 7			TUIII.		μι		
1	0.20	70.3	0.12		30.7	0.14		71.0	0.17		04.0	0.07
2		74.2	0.11	0.06	72.3	0.10		78.9	0.09		72.5	0.13
3		57.5	0.18	0.06	76.4	0.14		63.6	0.19		63.8	0.16
4		53.5	0.19		72.2	0.16		64.2	0.15		72.4	0.16
5		46.6	0.16		63.9	0.19		72.1	0.09		62.3	0.14
6		64.7	0.14	0.04	75.1	0.15	0.01	77.9	0.10		68.8	0.14
7		72.9	0.09	0.07	79.2	0.12	0.19	78.5	0.14	0.09	70.6	0.08
8		66.6	0.16	0.16	79.5	0.11		66.2	0.18		72.2	0.13
9		65.0	0.18	0.10	80.5	0.16		64.6	0.19	0.32	81.5	0.10
10	0.86	84.2	0.08		69.1	0.21		63.7	0.16		62.0	0.19
11		74.9	0.17		58.2	0.19		66.1	0.15		67.2	0.15
12	0.89	82.9	0.07		68.5	0.19	0.65	86.8	0.04	0.06	70.6	0.13
13	1.09	72.3	0.17		63.0	0.22		64.8	0.14		67.7	0.09
14	0.01	64.6	0.20		69.3	0.20		62.2	0.14		67.3	0.12
15		72.2	0.12	0.44	74.5	0.18		71.8	0.12	0.06	81.5	0.03
16	0.45	71.0	0.18	0.02	69.6	0.22		66.1	0.16	0.01	76.7	0.07
17	0.13	59.9	0.24		69.5	0.20		67.4	0.18		74.5	0.11
18		63.3	0.19		69.2	0.22		69.6	0.15		68.5	0.13
19		55.5	0.20	0.03	62.9	0.25		66.3	0.18	0.01	81.1	0.08
20		56.3	0.21		60.4	0.23		71.4	0.15	0.03	84.3	0.05
21		60.4	0.20		63.4	0.18		68.8	0.17		69.7	0.11
22		63.7	0.21	0.24	75.1	0.15	0.23	78.1	0.11		65.9	0.06
23		65.0	0.21	0.41	72.1	0.15		59.8	0.18		77.0	0.09
24	0.18	72.8	0.18		60.1	0.17		65.1	0.17		74.9	0.11
25	0.40	81.1	0.10		62.3	0.19		67.4	0.18		72.7	0.11
26		79.1	0.13	0.05	67.5	0.14		68.5	0.14		74.8	0.11
27	0.04	78.7	0.16	0.02	76.7	0.13	1.71	82.2	0.09		72.3	0.13
28	0.20	82.5	0.12	0.05	77.2	0.10	1.40	83.7	0.07		72.9	0.13
29		76.6	0.11	0.02	75.0	0.10		76.3	0.16	0.12	79.8	0.05
30		61.3	0.16		69.9	0.17	0.13	75.1	0.16		79.8	0.09
31		00	0.04	0.42	88.5	0.04		79.5	0.09			0.00

Table 8. 2013 Daily total precipitation, average relative humidity, and estimated potential evapotranspiration (PET).

^a Total precipitation (inches).
 ^b Average relative humidity (percent).
 ^c Estimated PET (inches per day).
 Source: Enviro-weather (http://www.agweather.geo.msu.edu/mawn/station.asp?id=htc)

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CHAPTER II:

CHARACTERIZATION OF VEGETATIVE COMPATIBILITY GROUP OF SCLEROTINIA HOMOEOCARPA ISOLATES FROM THREE CULTIVARS OF AGROSTIS STOLONIFERA AND THREE IRRIGATION REGIMES

Abstract

Many vegetative compatibility groups (VCG) of Sclerotinia homoeocarpa FT Bennett have been identified, with researchers reporting anywhere from 4 to 54 distinct VCGs. In this study, on each of two dates, a total of 180 isolates of S. homoeocarpa were recovered from a creeping bentgrass (Agrostis stolonifera L.) fairway at the Hancock Turfgrass Research Center in East Lansing, Michigan which was subjected to three different irrigation program treatments (infrequent nighttime, daily nighttime, or daily morning) and seeded to three different creeping bentgrass cultivars ('Declaration', L-93', or 'SRP-1WM'). Recovered isolates were scored for VCG based on barrage zone formation in an effort to determine if irrigation regime or creeping bentgrass cultivar corresponded to VCG prevalence. Results indicated that VCG B and F, averaging 45 and 33% overall, respectively, were the most predominant while no isolates of VCG A were recovered, and only 6% of isolates from one collection date were VCG D. VCG B was the most commonly recovered VCG for all irrigation regimes and all creeping bentgrass cultivars, followed by VCG F, except for the infrequent nighttime irrigation program on the second sampling date when VCG F isolates were more predominant than VCG B. Influence of the irrigation regimes or creeping bentgrass cultivars tested in this fairway trial in Michigan did not vary regarding selection of VCG in Agrostis stolonifera.

Introduction

Dollar spot (Sclerotinia homoeocarpa FT Bennett) is a major disease of warmand cool-season turfgrass (Smiley et al., 2005). Although numerous methods for control of dollar spot are available, the use of fungicides remains among the most reliable management strategy, causing dollar spot to be one of the most economically important diseases of turf (Vargas, 2005). Characteristic symptoms of dollar spot on golf course putting greens, tees, and fairways include blighted, tan-colored, sunken spots up to 5 cm in diameter, reaching up to 15 cm diameter in taller turf stands such as in golf course roughs, athletic fields and home lawns. Spots may coalesce, resulting in larger blighted areas. In the mornings when sufficient dew is present on the turfgrass, white, fluffy fungal mycelia may be observed. Leaf symptoms may begin with chlorotic, watersoaked lesions which become white to tan-colored, typically with reddish-brown margins. Typically, dollar spot is favored under conditions of high humidity and temperatures that range from 15 to 30°C. Dollar spot may occur for a majority of the growing season in the northern USA, usually from late spring through fall (Smiley et al., 2005; Vargas, 2005). The disease is thought to spread mainly by mycelial growth from soil and infected foliage to nearby plants and by direct transport of infected clippings on golf equipment or machinery. The taxonomy of the fungus is inconclusive due to the lack of a fertile teleomorph, which may be an indicator that populations are clonal and vegetative compatibility group (VCG) variability is a result of vegetative change during mitosis.

Various management strategies including chemical, cultural, biological, and host resistance, are employed to combat dollar spot, with chemical fungicide usage the most

common, particularly under high disease pressure conditions. Cultural management strategies for control of dollar spot have been studied. The impact of removal of dew on dollar spot by mowing with either a walking or triplex mower or pulling a floor squeegee over plots (Beard and Batten, 1982; Delvalle et al., 2011; Ellram et al., 2007) and lightweight rolling with a turfgrass rolling machine (Giordano et al., 2012) has been investigated. Additionally, the effect of fertility using various rates and types of fertilizers (Bloom and Couch, 1960; Landschoot and McNitt, 1997; Markland et al., 1969), avoidance of drought stress by applying various amounts of water (Couch and Bloom, 1960), and management of irrigation programs with both time of day and frequency of application of irrigation (Jiang et al., 1998; McDonald et al., 2006) have also been studied.

Creeping bentgrass (*Agrostis stolonifera* L.) is the most common cool-season turfgrass used on golf courses (Lyman et al., 2007). Cultivars of creeping bentgrass differ in susceptibility to *S. homoeocarpa* (Bonos et al., 2006; Casler et al., 2007; Cole et al., 1969; NTEP, 2010). Based on results from the National Turfgrass Evaluation Program (NTEP) in 2010, 'Declaration' and 'SRP-1WM' were among the least susceptible cultivars of creeping bentgrass available, while 'L-93' was more susceptible to dollar spot (NTEP, 2010). Although host resistance to dollar spot impacts disease development, the mechanism behind this resistance is not well-understood.

Isolates of *Sclerotinia homoeocarpa* can be categorized by vegetative compatibility group (VCG). Fungal strains are said to be vegetatively compatible if hyphal fusion occurs when the isolates are grown together and form a stable heterokaryon, in which case they are members of the same VCG. For this to occur, the

strains must possess identical alleles at particular sets of loci (Leslie, 1993). Hyphae that do not anastomose are considered incompatible. In an incompatible reaction, a barrage zone develops at the hyphal junction (Sonoda, 1989). Formation of a barrage zone can be characterized by the development of dark zones of pigment where non-compatible hyphae converge (Leslie, 1993). The central portion of the barrage zone contains dead or collapsing and degenerating cells as described by Newhouse et al. (1991), while either edge of the zone may contain dense, aerial mycelia (Leslie, 1993). Deng et al. (2002) described three incompatible reactions as gap, line-gap, and barrage. In a gap reaction, a 3-10 mm gap with two dark lines at the edges formed and was visible on the back of potato dextrose agar plate. A line-gap reaction was described as having a narrow gap 1 mm across with one or two dark lines on the underside of the media plate.

Sonoda et al. (1989) identified 54 distinct VCGs in accessions of *S. homoeocarpa* from Florida using barrage formation as an indicator of incompatibility, while Jo et al. (2008) regrouped 11 VCGs using barrage zone characterization to 5 VCGs using nitrate-nonutilizing (*nit*) mutants. Other researchers have reported differing numbers of VCGs in *S. homoeocarpa* (Chakraborty et al., 2006; Viji et al., 2004). In a study examining the response of bentgrass cultivars to isolates of *S. homoeocarpa* from ten different VCGs, Chakraborty et al. (2006) determined that bentgrass cultivars exhibited significant differences in dollar spot severity and that fungal isolates differed significantly in virulence. In Michigan, Powell and Vargas (2001) demonstrated that representatives of six *S. homoeocarpa* VCGs were present throughout the season from spring into fall, with two VCGs, denoted as VCG A and VCG B, identified as the most

predominant. Isolates of *S. homoeocarpa* VCG F were considered seasonal and were identified only in late summer epidemics for the first two years of the study; however, in the third year, isolates of *S. homoeocarpa* VCG F were only recovered in the early summer epidemics (Powell and Vargas, 2001). It is unclear whether this seasonal variation was due to environmental conditions or some other factor. Viji et al. (2004) identified a correlation between virulence and VCG in *S. homoeocarpa*, while Chakraborty et al. (2006) demonstrated that fungal isolates differed significantly in aggressiveness regardless of host cultivar or species of grass. This supports the idea that by using a few highly virulent fungal isolates, turfgrass breeders could select for resistance to a wide range of isolates.

The objective of this study was to determine whether a) different irrigation regimes and b) different creeping bentgrass cultivars, alone or in combination, influenced the population VCG characteristics of isolates of *S. homoeocarpa* recovered from field plots.

Materials and Methods

A field study was conducted at the Hancock Turfgrass Research Center on the campus of Michigan State University in East Lansing, MI. Overall, the effect of different irrigation programs and cultivars of creeping bentgrass (*Agrostis stolonifera* L.) on the appearance of vegetative compatibility group (VCG) of isolates of *Sclerotinia homoeocarpa* recovered from the experimental plots in Jul and Aug 2011 was examined. Three irrigation programs were situated in positions determined by statistically generated random allocations in 11 x 11 m plots and replicated three times. Irrigation programs investigated were daily irrigation at 0500 h (daily morning), daily irrigation at 2200 h (daily nighttime) or irrigation at 2200 h twice weekly (infrequent nighttime). Within the irrigation plots, three creeping bentgrass cultivars, 'Declaration', 'L-93', and 'SRP 1-WM', were seeded to 2.7 x 3.7 m split plots and replicated four times. Buffer strips surrounding irrigation plots were seeded to the DS-susceptible cultivar 'Crenshaw' (Figure 7.)

The study site received a total of 146 kg N ha⁻¹ from either 18-9-18 or 24-2-12 granular fertilizer monthly. The herbicide Mec Amine-D (2, 4-D, Mecoprop-p and Dicamba) (Loveland Products, Greeley, CO) was applied at 30 L ha⁻¹ on 21 Jun 2011 for the control of broadleaf weeds. Insects were controlled with carbaryl (Sevin; Bayer Environmental Science, Research Triangle Park, NC) on 21 Jul 2011, applied at 50 L ha⁻¹ for the control of black cutworms (*Agrotis ipsilon*).

Virulence of isolates of *S. homoeocarpa* was determined by center-point inoculation of 'A4' cultivar of creeping bentgrass (*Agrostis stolonifera* cv 'A4'). *S.*

homoeocarpa was grown on potato dextrose agar (PDA: 39 g PDA L⁻¹ water; Becton, Dickinson and Co, Sparks, MD). One 5 mm agar plug containing mycelia of *S. homoeocarpa* was placed in the center of each of two creeping bentgrass pots which were placed in quart-sized plastic bags containing 150 ml water. Bags were sealed and incubated at 22 ± 2 C for 10 days. All fungal isolates tested successfully infected 'A4' creeping bentgrass. Representatives of VCGs A-F, which were confirmed to be virulent, were used for the duration of the trial.

Plots were inoculated with S. homoeocarpa-infested sand-cornmeal topdressing mixture. The inoculum was prepared using a modified version of the method of Goodman and Burpee (1991). A mixture of silica sand and cornmeal (Quaker Oats Co., Chicago, IL) (2:1, v/v) and 2.4% potato dextrose broth (Becton, Dickinson and Co, Sparks, MD) (5% v/v) was placed in 15 x 25 cm aluminum baking pans, covered with two layers of aluminum foil and placed into a second aluminum baking pan. The sandcornmeal mixture was autoclaved at 121 C for 45 minutes. S. homoeocarpa on potato dextrose agar (PDA: 39 g PDA L⁻¹ water; Becton, Dickinson and Co, Sparks, MD) in 9 cm petri dishes was cut into 30-100 pieces and placed on the sterile sand-cornmeal. After incubation at 22 ± 2 C for 2 weeks, the media was chopped and kneaded by hand to break down clumps. This procedure was followed for each of six different VCG isolates (A, B, C, D, E, and F) of *S. homoeocarpa* which were previously collected from turfgrass in MI by Powell (1998). A mixture containing equivalent amounts of each VCG was blended together by hand for field application. On 17 Jun 2011, the blended sandcommeal inoculum was applied to field plots at a rate of 2.1 kg 100 m^{-2} using a Gandy lawn spreader (Model 36H13, Gandy Co, Owatonna, MN).

Dollar spot-infected leaf tissue was collected on 15 Jul and 25 Aug 2011. Twenty samples of symptomatic leaf tissue from each creeping bentgrass cultivar were randomly collected from every irrigation plot. Samples were stored in 5.7 x 8.9 cm coin envelopes (Quality Park Products, St. Paul, MN) at 1 - 4 C.

Figure 7. Aerial photograph of the field trial site. Irrigation plots are labeled with irrigation regime. Sub-plots within irrigation plots were established to either 'Declaration', 'L-93', or 'SRP-1WM' cultivars of creeping bentgrass.



To recover fungal isolates from collected leaf tissue, Individual blades with advancing disease margins were placed onto acidified water agar (24 g agar L^{-1} water with 10 ml lactic acid) and incubated at 22 ± 2 C for 3 days. After development of mycelia characteristic of *S. homoeocarpa* from the blades of grass, a 3 mm diameter agar plug from each fungal isolate was transferred to PDA (39 g PDA L^{-1} water).

Pairing to VCG for each isolate was performed on PDA plates amended with red food coloring (10 drops L⁻¹ water with 39 g PDA; McCormick and Co., Inc., Hunt Valley, MD) to help delineate barrage zone formation (Kohn et al., 1990). For each recovered isolate, a 2 mm mycelial plug was placed at the center of a food color amended PDA plate. For one sample plate, a plug of each of tester strains belonging to VCGs A-E were plated 3.5 cm apart surrounding the central sample plug. A second sample plate contained VCG F and the recovered isolate. Each plate also contained a pairing with the recovered isolate to ensure self-fusion and to act as a negative control indicated by the absence of barrage formation. Each recovered isolate and combination of tester isolates was plated three times. Compatibility of the test isolates with known VCG isolates was determined after 7 days of incubation at 22 C. Incompatible reactions were scored as the presence of dark barrage zones at the contact margins of cultures (Newhouse and MacDonald, 1991). Reactions were scored as compatible when hyphal growth was confluent. For reactions that were not readily resolved, the incubation period was increased to 3 weeks. For slow growing recovered isolates, in order to encourage adequate growth for VCG pairing and to avoid overgrowth of the recovered isolate with faster growing known VCG isolates, slow growing recovered isolates were re-plated 3 to

4 days prior to the addition of the tester isolates. This prevented faster growing isolates from overrunning slower growing isolates.

Results

Isolates were readily recovered from the acidified water agar plates after incubation. Mycelia were thin and sparse, but once transferred to PDA, cultures generally grew rapidly and filled the plate after incubation for 4 days. On PDA, mycelial growth appeared typical for *S. homoeocarpa*, and ranged from dense, fluffy and white to darker brown mycelia. Some recovered isolates grew very slowly with sparse mycelial growth, typically brownish in color. When being scored for vegetative compatibility, these isolates were incubated for 3-4 days prior to the addition of tester strains onto the scored agar plate in order to allow sufficient growth and avoid being overrun by faster growing tester isolates.

After incubation, vegetative compatibility of recovered specimens was generally easy to determine. All self-fusion reactions were confluent and served as an example of a compatible reaction on every plate. Incompatible reactions were identified based on the formation of dark barrage zones or the presence of abundant, dense, aerial mycelia at the junction of isolates (Figure 8). For each collection date, the overall percentage of the total number of specimens recovered is listed by VCG (A-F) in Table 9. The number of specimens classified within each VCG followed the same trend on both collection dates except for VCG D, which was not recovered on 15 Jul. Vegetative compatibility group B was the most prevalent, recovered at 47 and 42% on 15 Jul and 25 Aug, respectively, followed by VCGs F, E and C. VCGs B and F accounted for 78% of the VCGs of the collected fungal specimens. No isolates were scored as compatible with VCG A. On 15 Jul, none of the recovered specimens exhibited compatible reactions with VCG D, while on 25 Aug, only about 5% were identified.

Figure 8. Vegetative compatibility assay among isolates of *Sclerotinia homoeocarpa* on potato dextrose agar amended with red food coloring after 7 days. The center isolate in each plate is the recovered sample and is surrounded by VCGs A-E (clockwise from top). Recovered samples were tested for compatibility with VCG F on separate plates. Incompatible reactions are identified by the formation of a dark or dense barrage zone. "S" denotes the position of the recovered sample used to confirm self-fusion and as a negative control on each plate. "C" indicates a compatible reaction. Plate A was scored as compatible with VCG E, Plate B with VCG B, and Plate C was not compatible with VCGs A-E tested here.



Table 9. Percentage of total number of *S. homoeocarpa* isolates recovered for each VCG from irrigation x cultivar experiment.

Sampling	Frequency of recovery of VCG groups of S. homoeocarpa (%)								
Dale	A*	В	С	D	E	F			
15-Jul-11	0	47	10	0	13	30			
25-Aug-11	0	42	7	6	9	36			
Average	0	44.8	8.5	2.8	10.3	33.2			

* VCGs A, B, C, D, E, and F are named as per Powell and Vargas (2001).

VCG B isolates were predominant on all cultivars at both dates followed by VCG F, except for 'L-93' when, on 25 Aug, VCGs B and F were recovered in equivalent frequencies (Table 10). On 15 Jul, less than 18% of the isolates were identified as either VCG C or E. On 25 Aug, VCGs C, D, and E were identified between 3 and 13% of the time. The distribution of VCG pairing of recovered isolates was similar among cultivars.

Percentages of isolates in each VCG by irrigation regime are listed in Table 11. Data from both daily irrigation treatments (morning and nighttime) follow the same trends as for the effect of cultivar. VCGs B and F were recovered more frequently from each irrigation regime treatment than any other VCG. However, samples recovered from the plots that were irrigated infrequently at night resulted in 58 and 31% VCG B and F, respectively on 15 Jul while they were 33 and 47% VCG B and F, respectively on 25 Aug. This was the only time VCG B was not the most prevalent isolate recovered.

Table 10. Frequency of Vegetative Compatibility Group (VCG) of *Sclerotinia homoeocarpa* isolated from creeping bentgrass under three irrigation regimes from the Hancock Turfgrass Research Center (East Lansing, MI) in 2011.

	Frequency of Vegetative Compatibility Groups (VCG) of Sclerotinia homoeocarpa (%)											
	Creeping Bentgrass Cultivar ^a											
		15-Jul-11 ^b			25-Aug-11							
VCG ^C	'Declaration'	'SRP-1WM'	'L-93'		'Declaration'	'SRP-1WM'	'L-93'					
А	0	0	0	-	0	0	0					
В	49	46	48		39	46	42					
С	14	8	7		11	7	4					
D	0	0	0		8	3	6					
E	6	14	18		13	8	6					
F	31	32	28		29	37	42					
SEM ^d	8.0	7.6	7.7		5.9	8.0	8.1					

^a Creeping bentgrass cultivars were irrigated at 0500 h daily, 2200 h daily, or 2200 h twice weekly.

^b Date of sample.

^c VCGs A, B, C, D, E, and F are named as per Powell and Vargas (2001).

^d SEM = Standard Error of the Mean per cultivar (N=9).

Table 11. Vegetative compatibility group (VCG) of *Sclerotinia homoeocarpa* isolated from creeping bentgrass using three irrigation programs at the Hancock Turfgrass Research Center (East Lansing, MI).

	Frequency of Vegetative Compatibility Groups (VCG) of Sclerotinia homoeocarpa (%)											
			Irrigation	Regime ^a								
-		15-Jul-	11 ^b		 25-Aug-11							
-	0500 h	2200 h	2200 h Twice	0500 h	2200 h	2200 h Twice						
VCG	Daily	Daily	Weekly	Daily	Daily	Weekly						
А	0	0	0	0	0	0						
В	41	43	58	40	53	33						
С	13	9	7	9	6	8						
D	0	0	0	10	4	7						
Е	16	18	4	8	9	6						
F	30	30	31	33	28	47						
SEM ^d	6.7	7.0	9.5	6.5	8.3	7.6						

^a Samples collected from 'Declaration', 'L-93', and 'SRP-1WM' cultivars of creeping bentgrass were combined for each irrigation program (0500 h daily, 2200 h daily, and 2200 h twice weekly).

^b Date of sample.

^c VCG A, B, C, D, E, and F are named as per Powell and Vargas (2001).

^d SEM = Standard Error of Mean per irrigation regime (N=9).

Discussion

The results of this study indicate similarity in the association among the irrigation regimes or creeping bentgrass cultivars tested to a particular VCG of *Sclerotinia homoeocarpa*. The majority (78% of total) of recovered isolates were classified as either VCG B or F, regardless of irrigation program or creeping bentgrass cultivar. On the 15 Jul collection date, nearly 47% of all isolates collected paired with VCG B, followed by 30% with VCG F. Similarly, on the 25 Aug collection date, isolates expressing compatible reactions with VCG B numbered 42% of the total collected while 36% were compatible with VCG F. Powell and Vargas (2001) found VCG A to be the most predominant of the *S. homoeocarpa* isolates recovered in MI, followed by VCG B. Although the current study did not recover specimens belonging to VCG A, the findings support those of Deng et al. (2002), Viji et al. (2004), and DeVries (2006) who demonstrated that VCG B was predominant in the populations that were investigated.

Although VCGs A-F were introduced as initial inoculum in equivalent amounts as field inoculum for the trial, not all of the VCGs were recovered at either evaluation date. It is possible that some VCGs, such as VCGs B and F, were more virulent than others. Viji et al. (2004) classified *S. homoeocarpa* isolates into four virulence groups based on DS severity: highly, moderately, and weakly virulent and avirulent. Certain virulence groups corresponded to specific VCGs; however, this was not confirmed upon AFLP analysis Viji et al. (2004). VCG B was comprised of isolates in all four virulence group classifications. Contrary to findings of the current study where no VCG A isolates were recovered, VCG A isolates were highly virulent in the Viji et al. (2004) study. All of the *S. homoeocarpa* VCG tester isolates used for field inoculation in this study were found to

be virulent prior to field inoculations, but the level of virulence as described by Viji et al. (2004) was not determined.

In examining isolate recovery by creeping bentgrass cultivar, VCG B was predominant, followed by VCG F, except at the 25 Aug sampling date from the cultivar 'L-93' from which VCGs B and F were recovered in equivalent amounts. VCGs B and F represented 75 to 80% of the isolates recovered on 15 Jul and 68 to 84% from 25 Aug. These results indicated that the most predominant VCGs were recovered from all of the cultivars and no VCG corresponded to a specific cultivar. Similarly, when evaluating isolate recovery by irrigation regime, VCG B followed by VCG F were most prevalent, except in the infrequent nighttime irrigation program plots on 25 Aug when VCG F was more prevalent than VCG B isolates. This was the only occurrence when VCG B was not the most frequently recovered isolate. Even though the trend from the first to second collection reversed for the infrequent nighttime irrigation program, 89 and 80% of recovered isolates were represented by a combination of VCGs B and F for each date, If further collections had been made, perhaps on an annual basis respectively. throughout the study, a better understanding of the potential impact of infrequent irrigation on VCG of S. homoeocarpa could have been elucidated.

It may be possible that *S. homoeocarpa* VCGs could be favored by certain environmental conditions, such as soil moisture level or the timing of irrigation applications to turf, which may be affected by irrigation programming since no VCG A isolates were recovered in this study. If irrigation patterns which favor less virulent isolates of *S. homoeocarpa* could be identified, this could lead to another means of reducing DS pressure under field conditions. Irrigation programming could then be

patterned to favor less virulent isolates of *S. homoeocarpa*. When examining VCGs of recovered specimens of *S. homoeocarpa* from fairway plots subjected to one of three different irrigation regimes, each containing creeping bentgrass cultivars 'Declaration', 'L-93', and 'SRP-1WM', VCG recovery was similar for each irrigation regime or cultivar.

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CONCLUSIONS

Chapter 1: Irrigation regime and cultivar field study

Results of this research indicated that irrigation regime and host resistance impact dollar spot (DS) of creeping bentgrass on fairway turf in MI. In 2011, 'SRP-1WM' watered at 2200 h twice weekly (infrequently), 0500 h daily, or 2200 h daily exhibited significantly less DS than 'Declaration' watered in the same manner. 'L-93' developed significantly more DS compared to 'Declaration' when each cultivar was irrigated infrequently or daily. These data demonstrate the importance of host resistance with regard to DS incidence when irrigation programming is comparable. When irrigated at 2200 h twice weekly, all cultivars developed significantly more DS than when irrigated at 2200 h daily with similar total weekly amounts of applied water ha⁻¹. Irrigating with approximately the same weekly volume of water at 2200 h on a daily basis yielded significantly less DS than on an infrequent basis regardless of host cultivar. Watering at 2200 h daily compared to 0500 h daily yielded significantly less DS for 'L-93' and 'Declaration', however, for 'SRP-1WM', daily irrigation treatments did not significantly affect DS incidence.

Data from 2013 supported the findings from 2011. For the main effect of irrigation, 2200 h daily irrigation averaged over all cultivars for the season exhibited significantly less DS than either 0500 h daily or 2200 h twice weekly irrigation programming. Both daily irrigation regimes developed significantly less DS than the infrequent irrigation program on a seasonal basis when averaged for all cultivars. There are several explanations which may account for this difference in impact on DS resulting from irrigation programming. It is possible that under conditions of daily irrigation, soil

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moisture content was maintained at a consistently higher level when compared with infrequent irrigation, even though the same weekly volumes were applied. The infrequent applications may have wet the soil to a deeper level within the soil profile at the time of application, but then may have resulted in the top 1 - 2 cm drying down between irrigation events.

Another consideration is that by watering daily, the leaf wetness duration may have been affected. Dew formation and its duration on plant leaves may have been altered (ie diluted or displaced) daily as compared with infrequent programming which would only affect the dew twice weekly. It is unknown whether the interruption of dew formation, as would occur at the 2200 h irrigation event, or its displacement at 0500 h, would influence pathogen growth. By slowing or interfering with fungal growth, reduced disease incidence could result.

Yet another consideration is that by supplying irrigation to the plants daily rather than allowing the soil to dry between irrigation events, plant health could be affected. In reducing potential plant stress which could result from drying, maintaining more consistent soil moisture might allow plants to focus energy on disease resistance rather than physiological processes associated with drought stress.

By combining the use of host resistance and irrigation management, a reduction in DS of creeping bentgrass may be achieved. This combination of cultural practices may be of importance in areas where DS is problematic. Further evaluation of the mechanism regarding soil moisture, disease development and impact on microbial populations is needed. Additionally, further research examining the role of guttation

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water, canopy humidity, and leaf wetness duration may enhance current knowledge regarding the *S. homoeocarpa* infection process, leading to a better understanding of parameters that may enhance or inhibit infection of turfgrass.

Chapter 2: Vegetative compatibility group study

Vegetative compatibility group (VCG) pairings indicate similar correspondence of VCG with host resistance or irrigation regime. Isolates recovered from samples collected were predominantly paired with VCG B followed by VCG F for every cultivar and every irrigation regime. The exceptions were from the 25 Aug sampling when equal numbers of VCGs B and F were recovered in the 'L-93' plots. On that same date, samples from infrequent nighttime irrigation programming yielded more VCG F than B. This was the only occurrence when VCG B was not the most prevalent compatibility type isolated. Attempts by other researchers have been made to correlate VCG among *S. homoeocarpa* accessions to fungicide resistance, virulence, geographic distribution, host, and seasonal epidemics. Although there have been a few reported successes in correlating VCGs to studied factors, the association of VCG in *S. homoeocarpa* with clearly defined factors has largely eluded researchers.

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