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## EFFECTS OF DEW REMOVAL AND TRINEXAPAC-ETHYL ON FUNGICIDE EFFICACY FOR DOLLAR SPOT CONTROL

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Agronomy

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#### ABSTRACT

Dollar spot, caused by the pathogen *Sclerotinia homoeocarpa* F.T. Bennett, is a disease of all turfgrass species and is considered the most economically important disease on golf courses. Many cultural and chemical management practices are necessary to reduce the damage caused by this disease. Canopy moisture (e.g., leaf wetness) plays an important role in the development of dollar spot and routine removal of dew has been shown to reduce disease severity. The effect of canopy moisture on fungicide efficacy at the time of fungicide application, however, is not well understood. In addition to management practices directly related to the reduction of dollar spot, other inputs like the application of plant growth regulators are common to golf courses for maintaining high quality turf. Trinexapac-ethyl (TE) is one of the most commonly used PGRs used on golf courses, but its influence on dollar spot is unclear. The objective of this field study was to elucidate the influence of dew removal methods at the time of fungicide application on dollar spot control within turfgrass regulated by TE. Field studies were initiated at the Joseph Valentine Turfgrass Research Center on a mature 'Penneagle' creeping bentgrass (Agrostis stolonifera L.) fairway. Main factors in the study included three dew removal strategies (untreated, rolled and mowed) prior to the application of fungicides (untreated, chlorothalonil, propiconazole and iprodione). All treatments were applied to turfgrass previously treated with TE treatments (untreated and TE). Dollar spot infection centers (DSIC) and area under the disease progress curve data were analyzed using the PROC MIXED procedure in SAS. The presence or absence of dew at the time of fungicide application had no influence on fungicide efficacy. The effect of regulation by TE on fungicide-treated turf had little influence on dollar spot severity. However, the effect of regulation on non-fungicide treated turf resulted in a significant reduction in DSIC. Based on the results of this study, dew

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removal prior to the application of fungicides in the morning is an unnecessary step and does not influence fungicide efficacy. Although TE had little influence on disease severity where fungicides were applied, its use in areas where fungicide applications are not possible may provide a small, but beneficial reduction of dollar spot.

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#### LITERATURE REVIEW

#### Introduction

Dollar spot, caused by *Sclerotinia homoeocarpa* F.T. Bennett, is a foliar disease that affects most turfgrass species. Although all species are susceptible, disease severity varies among cultivars and species (Hsiang, 1995; Doney, 1994; Myer and Smejkal, 1995; Bonos, 2005). Dollar spot is a widespread, problematic disease on golf courses throughout North America, Central America, Australia, New Zealand, Japan, the British Isles, and continental Europe (Fenstermacher, 1980; Vargas, 2005). More money is spent managing dollar spot than any other turfgrass disease (Vargas, 2005).

In the mid-Atlantic region of the United States, dollar spot is generally active from late spring to late fall when temperatures range from 15 to 30°C (Smiley et al., 2005). On closely mown turf, dollar spot appears as small, round, bleached-out or brown, sunken patches that typically range in size from 1 to 3 cm (Couch, 1995; Vargas, 2005). Individual infection centers seldom exceed 5 cm in diameter, but spots may coalesce into large, irregular patches if the disease becomes severe (Smiley et al., 2005; Vargas, 2005). Single or multiple lesions may develop on individual leaves and lesions can expand across the entire leaf margin. Infected leaves may initially appear chlorotic, and then become water-soaked, until finally turning bleached-out or straw colored (Smiley et al., 2005). In severe situations, the uniformity of the turf surface may be reduced and the disease ultimately may lead to plant death and weed encroachment (Burpee, 1997; Smiley et al., 2005; Vargas, 2005).

White cobweb-like mycelium of the pathogen often can be seen within spots of infected turf during the morning hours. Mycelium can move from infested to healthy leaves in close proximity or be physically distributed through infested clippings (Monteith and Dahl, 1932; Vargas, 2005). *S. homoeocarpa* can infect leaves via indirect penetration when mycelium enters the plant through wounds, cuttings and natural plant openings such as stomata or hydathodes (Monteith and Dahl, 1932; Endo, 1966). Appressoria have also been observed suggesting direct penetration into the leaves (Endo, 1966). *S. homoeocarpa* does not infect roots directly, but has been found to produce root-damaging mycotoxins (Malca and Endo, 1965).

On artificial media (e.g., potato dextrose agar), *S. homoeocarpa* is characterized by prolific aerial mycelia that can engulf a petri dish within 3-5 days (Monteith and Dahl, 1932). Hypha are septate and usually exhibit a characteristic y-angled branching pattern. After two to three weeks of initial growth on nutrient media, a dark sclerotized region, commonly referred to as stroma appears around the original source of inoculum. Stroma is formed by conversion of superficial hyphae of the mycelium into small thick-walled cells (Bennett, 1937).

*S. homoeocarpa* may be capable of surviving in plant debris as a facultative saprophyte for brief periods (Vargas, 2005). The pathogen is believed to overwinter as darkly pigmented stromata along the margin of dollar spot lesions or survive as dormant mycelium within infested grass tissues and crowns (Fenstermacher, 1980; Couch, 1995; Smiley et al., 2005). The teleomorph of *S. homoeocarpa* is uncommon and spores are rarely observed in nature (Baldwin and Newell, 1992; Smiley et al., 2005). The basic biology of *S. homoeocarpa*, including the form in which it exists when it is not causing disease, remains unknown (Harman et al., 1999). Harman et al. (1999) proposed the possibility of two stages of the pathogen's life cycle: a slow-

growing quiescent phase and a pathogenic aggressive phase. Detection of the quiescent phase in areas where the pathogen resulted in turf death, however, has not been successful.

#### Taxonomy

The taxonomic status of *S. homoeocarpa* remains controversial since the first report of the fungus (Rossman, 1987). The pathogen was first misidentified as a *Rhizoctonia* species based on its similarities to *Rhizoctonia solani* Kuhn, and was designated "small brown patch" (Monteith and Dahl, 1932; Bennett, 1937). The name of the disease was later changed to dollar spot in order to avoid confusion, and the pathogen was formally described as *S. homoeocarpa* in 1937 (Monteith and Dahl, 1932; Bennett, 1937). The pathogen was also assigned into three distinct strains including the perfect British strain, the ascigerous British strain and the non-sporulating strain. The correct taxonomic identity has been questioned on many occasions and many have proposed that the correct genus of the dollar spot fungus be reclassified as a *Rustroemia, Lanzia* or *Moellerodiscus* species (Whetzel, 1945; Jackson, 1973; Kohn, 1979a; Baldwin and Newell, 1992; Carbone and Kohn, 1993; Holst-Jensen et al., 1998; Powell and Vargas, 2007). It has also been considered that more than one species might be involved in dollar spot epidemics (Jackson, 1973; Kohn, 1979b; Smiley et al., 2005). Despite these findings, the taxonomic classification of dollar spot remains in question.

#### **Environmental factors**

Several factors including temperature, soil and leaf moisture, and relative humidity (RH) contribute to *S. homoeocarpa* growth and infection of turfgrass. Bennett reported optimum

temperatures for mycelia growth to be between 20 to 25°C for the British strains and 30°C for the American strains (Bennett, 1937). Endo (1963) determined the cardinal temperatures for mycelia to be 5 to 32°C, with optimal growth occurring at 27°C on potato dextrose agar (PDA). He also found that infection occurs at temperatures between 15 and 27°C (Endo, 1963). Peak growth rates and maximum pathogenicity were reported to occur when temperatures are between 21 and 27°C and atmospheric humidity is >85% (Endo, 1963).

Several forecasting models have been proposed and developed based on various environmental variables including temperature and RH. In the Mills and Rothwell model (1982), fungicide applications are recommended when maximum air temperature (AT) is  $\geq$ 25°C and maximum RH is  $\geq$ 90% during any three days of a seven-day period. Burpee and Goulty (1986) and Walsh (2000), however, reported that when the Mills and Rothwell model was evaluated on putting greens in Canada, it over predicted dollar spot activity and resulted in unnecessary fungicide applications. A separate model developed by Hall called for fungicide applications following two consecutive days of rainfall and a mean air temperature of  $\geq$ 22°C or three consecutive days of rainfall and a mean AT of  $\geq$ 15°C (Hall, 1984). Burpee and Goulty (1986) and Walsh (2000) again found that the Hall forecasting model was ineffective as it under predicted dollar spot epidemics.

Soil moisture also influences dollar spot severity. Disease severity is generally enhanced on turf maintained under water stressed soil conditions versus turfgrass that is well irrigated (Couch and Bloom, 1960; Couch, 1966; Jiang et al., 1998; Vargas, 2005; Smiley et al., 2005; McDonald et al., 2006).

The amount of leaf moisture and leaf wetness duration also have been shown to play an important role in pathogenic fungi occurrence including *S. homoeocarpa*, as it requires free

water on the leaf surface to produce aerial mycelium and infect host tissue (Huber and Gillespie, 1992; Williams et al., 1996; Uddin et al., 2002). Dew primarily consists of condensed moisture from the atmosphere and guttation water. On cool nights following warm days, atmospheric moisture close to grass leaf blades usually condenses at a rate greater than it can evaporate, resulting in dew formation on the leaf surface. Guttation water is rich in carbohydrates and amino acids that are exuded from grass blades through hydathodes when turgor pressure builds up within the plant (Vargas, 2005). Exudates from creeping bentgrass comprise approximately one-third of the dew accumulation (Williams et al., 1998). Prolonged leaf wetness and nutrients in guttation fluids are believed to favor pathogen growth and facilitate infection by pathogenic microorganisms (Curtis, 1943; Curtis, 1944; Ivanoff, 1963; Goatley and Lewis, 1966; Marion, 1974). Dew present on the plant surface also enhances the pathogen's ability to adhere to the leaf surface, which further helps the pathogen to resist displacement by flowing water (Agrios, 2005).

#### **Cultural control:**

Various cultural practices have proven effective for suppressing or reducing dollar spot severity. Deep infrequent irrigation is mostly preferred and performed by numerous golf courses for playability and agronomic reasons including promotion of a deep root system and reducing disease potential (McDonald et al., 2006; Vargas, 2005; Smiley et al., 2005). Maintaining adequate soil moisture levels, however, has been shown to reduce dollar spot severity when compared to soils with lower soil moisture (Couch and Bloom, 1960; Couch, 1966; Jiang, et al., 1998; McDonald et al, 2006). In a greenhouse study, Couch and Bloom (1960) evaluated the effect of nutrition, pH, and soil moisture on *S. homoeocarpa* on Kentucky bluegrass (*Poa pratensis* L.). Disease severity was lower when soil moisture was maintained at field capacity

when compared to soil moisture maintained below 75% of field capacity. In one year of a 2-year field study on fairway height perennial ryegrass (*Lolium perenne* L.), Jiang et al. (1998) reported fewer dollar spot infection centers when turf was irrigated daily when compared to irrigation set to replace 80% of the evapotranspiration (ET). McDonald et al. (2006) reported dollar spot severity was negatively correlated with volumetric soil moisture in a 3-year field study on creeping bentgrass and perennial ryegrass. Enhanced dollar spot suppression from turf treated with chlorothalonil, paclobutrazol and a wetting agent (Primer, select, polymeric polyoxyalkylene 95% oxoalkonyl hydroxyl polyoxlalkanediyl 5%;) was found to be associated with soil moisture levels above 0.25 cm<sup>3</sup> cm<sup>-3</sup> (McDonald et al., 2006). On the other hand, Watkins et al. (2001) reported no significant differences in dollar spot severity between turf irrigated daily at 100% or scheduled to replace 60-80% ET.

Maintaining sufficient fertilization, especially nitrogen (N) has also been shown to suppress dollar spot (Huber, 1980; Nelson and Craft, 1992; Liu et al., 1995; Landschoot and McNitt, 1997; Golembiewski and Danneberger, 1998; Smiley et al., 2005; Agrios, 2005). Dollar spot severity was found to be correlated with turf color, and turf color was correlated with percent foliar N, indicating disease severity decreased as nitrogen availability increased (Landschoot and McNitt, 1997). Endo (1966) suggested that an available food base is essential for *S. homoeocarpa* growth and appressorium formation; thus, N deficient plants are more likely to develop senescent foliage that is more susceptible to infection when compared to plants with adequate N. Nitrogen is also an essential element for the production of many compounds including phenolics, phytoalexins, growth hormones, cellulose, and carbohydrates which are involved in host resistance (Huber, 1980; Agrios, 2005). Sufficient N applications also promote turfgrass recovery from disease injury and may allow the turf to outgrow the pathogen (Monteith

and Dahl, 1932; Couch, 1995; Liu et al., 1995). Studies have shown some natural and composts fertilizers were highly suppressive to dollar spot and the modes of action are assumed to be the increased microbial populations or increased nutrient supplement, but the actual suppressive mechanism is not well understood (Nelson and Craft, 1992; Boulter et al., 2000). Other studies have shown synthetic N fertilizers provide equal or better suppression of dollar spot disease than fertilizers consisting primarily of natural organic products (Liu et al., 1995; Landschoot and McNitt, 1997). Davis and Dernoeden (2002) reported dollar spot suppression to be associated more with N availability rather than enhanced microbial activity.

Various studies have shown that dew removal in the early morning hours can effectively reduce dollar spot severity (Williams et al., 1996; Nikolai et al., 2001; Ellram et al., 2007). Interrupting prolonged periods of leaf wetness may be accomplished via poling, mowing, through the application of light irrigation, and pruning or removing trees and shrubs to increase air circulation (Walsh et al., 1999). Mowing is one of most common cultural practices on a golf course and is an effective dew-removal method. Although wounds from cutting may facilitate infection by pathogenic fungi, early morning mowing has been shown to reduce disease severity because it displaces dew, reduces the duration of leaf wetness, disrupts fungal development, and removes infested clippings which potentially may serve as a source of secondary inoculum (Williams et al., 1996; Walsh et al., 1999, Smiley et al., 2005). Williams et al. (1996) reported that dew displacement by mowing or using a mower with the reels disengaged lowered dollar spot severity. Collecting or leaving clippings, however, did not consistently impact the severity of dollar spot. Ellram et al. (2007) found dew displacement at 0400h resulted in less dollar spot when compared to dew removal at 1000h or 2200h. The authors also concluded that daily

removal of dew more effectively reduces dollar spot severity than when dew is removed on alternate days (Ellram et al., 2007).

Lightweight rolling following early morning mowing has also been shown to significantly reduce dollar spot (Nikolai et al., 2001). The authors suggested that light weight rolling immediately following an early morning mowing may further disperse concentrated guttation fluid. Giordano et al. (2012), however, reported that rolling putting greens can significantly reduce dollar spot regardless of the time of day or presence of dew. Their results indicated that rolling may contribute to greater water-holding capacity in the upper root zone of the turfgrass canopy and suggested that this may aid in reducing dollar spot. Increase of water retention by rolling was previously reported by Nikolai et al. (2001) and the negative correlation of dollar spot severity and soil moisture concurs with previous findings by McDonald et al. (2006).

Mowing practices and dew removal strategies associated with fungicide performance on dollar spot control have been recently evaluated (McDonald et al., 2006; Putman and Kaminski, 2011; Pigati et al., 2010; Delvalle et al., 2011). Putman and Kaminski (2011) found that mowing frequency (e.g. 2, 4, or 6 days week<sup>-1</sup>) had no influence on fungicide performance. However, in the absence of fungicides, less frequently mowed turf (2 day week<sup>-1</sup>) resulted in less dollar spot than turf mown 4 or 6 days week<sup>-1</sup>. In another study, Delvalle et al. (2011) reported that dollar spot control with fungicides can be extended by daily dew removal or increased mowing frequency. Increasing the mowing frequency likely results in a reduction of dollar spot due to dew removal and/or physical disruption and removal of inoculum (Pigati et al., 2010; Delvalle et al., 2011).

Golf course superintendents often apply pesticides early in the morning to reduce the potential of pesticide exposure and avoid interfering with play. During the early morning, a significant amount of dew may be present on the surface. Information related to the effect of the presence of dew at the time a fungicide is relatively limited. McDonald et al. (2006) reported no significant differences in dollar spot severity when fungicides were applied in the morning with dew present versus dew displaced, but found that chlorothalonil occasionally provided better disease control when applied in the morning with dew displaced or at noon to a dry canopy when compared to morning applications with dew present. They suggested that increased chlorothalonil performance in the absence of dew is possible because increased quantities of chlorothalonil would adhere to the foliage and/or would not become diluted by free moisture. In a recent study, morning mowing prior to fungicide application (chlorothalonil, boscalid, iprodione, and propiconazole) improved the performance of all fungicides compared to plots mowed in the afternoon (Pigati et al., 2010).

Other practices such as raising mowing height, application of soil amendments, and/or maintaining adequate soil moisture may also promote quicker recovery from disease and allow the grass to outgrow the pathogen (Endo, 1966; Liu et al., 1995; Landschoot and McNitt, 1997; Boulter et al., 2000; Vargas, 2005; Turgeon, 2008). Cultural practices can become important management tools if they can be timed to coincide with disease outbreaks or chemical control practices, and may allow for a reduction in pesticide use (Landschoot and McNitt, 1997; Boulter et al., 2000).

#### **Chemical Control:**

Despite the ability of cultural practices to suppress dollar spot, fungicides are commonly required to provide adequate disease suppression on highly maintained turfgrass areas like those found on golf courses (Goodman and Burpee, 1991). A wide range of fungicides are available for the control of dollar spot. The variations in the sensitivity of the pathogen to different fungicides as well as the fungicide's mode of action play an important role in the length and level of control (Kohn et al., 1991; Smiley et al., 2005; Jo et al., 2006; Putman and Kaminski, 2010). A careful selection of fungicide products is essential for a successful dollar spot management program.

Select contact fungicides can effectively suppress dollar spot by targeting multiple sites of the pathogen. For this reason, *S. homoeocarpa* is generally less likely to develop resistance to these fungicides. Contact fungicides are often referred to as protectant fungicides because when applied, a protective-surface barrier is formed and remains on the outside of plant tissue which protects the plant from new infection. The length of activity of contact fungicides is generally short ( $\leq$ 14 d) due to exposure to rain, traffic, mowing, UV light, erosion and degradation of fungicidal compounds (Latin, 2011). Widely used contact fungicides include chlorothalonil, mancozeb, ethazol, maneb, quintozene (PCNB) and thiram (Smiley et al., 2005).

Penetrant or systemic fungicides are generally site-specific compounds that disrupt a single metabolic processes or structural site within the targeted pathogen (Latin, 2011). These fungicides can enter and move within the plant to varying degrees. Since they enter the plant, penetrant or systemic fungicides are less prone to environmental loss or degradation, and therefore generally have longer residual effects ( $\geq$ 21 d) than contact fungicides (Latin, 2011).

Based on the mobility of the active ingredient within the plant, these types of fungicides can be grouped as localized penetrants, acropetal penetrants and true systemic penetrants. Localized penetrants permeate the plant leaf and stay within or near the area on which it was deposited. Acropetal penetrants enter the plant and move upward in plant's xylem from the point of entry. True systemic penetrants can move both upward in the xylem and downward in the phloem after entering the plant.

The repeated use of several classes of fungicides has led to the development of resistant populations of *S. homoeocarpa*, resulting in partial or complete loss of fungicide efficacy (Detweiler et al., 1983; Golembiewski et al., 1995; Burpee, 1997; Gilstrap, 2005; Jo et al., 2006; Bishop et al., 2008; Koch et al., 2009; Putman and Kaminski, 2010). Tank-mixing multiple chemicals with differing modes of action or rotation of fungicides is widely recommended to manage dollar spot while minimizing the potential for resistance development (Gilstrap, 2005).

Plant growth regulators (PGRs) have become a conventional golf course turf management practice to regulate turfgrass growth, suppress certain weeds, reduce mowing frequency and clipping yield, and enhance turfgrass color, quality and density (Watschke et al., 1992). Select PGRs have been shown to suppress dollar spot and/or enhance fungicide efficacy (Burpee et al., 1996; Fidanza et al., 2006). Trinexapac-ethyl (TE), paclobutrazol and flurprimidol are the most commonly used PGRs to suppress turfgrass growth on golf courses in the United States. This group of PGRs regulates plant growth by inhibiting cell elongation in the gibberellic acid biosynthesis pathway (Burpee et al., 1996; Fidanza et al., 2006). Flurprimidol and paclobutrazol are fungistatic to *S. homoeocarpa* and have been shown to suppress dollar spot when applied alone or in combination with effective dollar spot fungicides (Burpee et al., 1996; Fidanza et al., 2006).

Unlike the fungistatic effects of flurprimidol and paclobutrazol, TE is considered to be less likely to affect dollar spot severity or fungicide performance (Burpee et al., 1996; Stewart et al., 2007). Several studies, however, have reported that TE improved grass tolerance to abiotic stress and stimulate non-fungistatic mechanisms which may contribute to disease suppression (Burpee et al., 1996; Golembiewski and Danneberger, 1998; Zhang and Schmidt, 2000; McCann and Huang, 2007; Xu and Huang, 2010).

Results from research on the influence of TE on dollar spot severity, however, are inconsistent. Field research findings have described neutral, beneficial or negative effects of TE on fungicide performance for the suppression of dollar spot (Golembiewski and Danneberger, 1998; Zhang and Schmidt, 2000; Fidanza et al., 2006, Stewart et al., 2008; Putman and Kaminski, 2011). Burpee et al. (1996) found that when TE was applied alone, it had no significant effect on dollar spot. When applied in combination with chlorothalonil, iprodione and propiconazole, however, TE enhanced fungicide efficacy in one year of the two-year study. Kaminski and Putman (2011) reported that TE had no influence on dollar spot when applied in combination with fungicides. In the absence of fungicides, however, TE has been shown to significantly suppress the disease (Golembiewski and Danneberger, 1998; Putman and Kaminski, 2011). In a separate study conducted by Stewart et al. (2008) on creeping bentgrass, TE rarely influenced dollar spot severity or fungicide performance when applied prior to the onset of dollar spot symptoms. When applied curatively, however, turf recovery from dollar spot damage following applications of chlorothalonil and propiconazole was significantly delayed within TEtreated plots in some occasions. The authors suggested that diminished turf growth may reduce fungicide uptake and limit the suppressive effect of the active ingredient, thereby delaying recovery from the outbreak.

Although there is information for dollar spot control related to dew removal strategies alone or following fungicide applications, the impact of dew present at the time of fungicide application is still not well understood. Additionally, studies related to the influence of TE on dollar spot have provided inconsistent results. The interaction of dew removal strategies at the time of fungicide application to turf under regulation by TE is important to consider. Therefore, the objectives of this research are to: i) evaluate the residual effectiveness of fungicides from a single preventive application on dollar spot control as influenced by dew removal at the time of application, and ii) determine the effect of turfgrass regulation by TE on residual effectiveness of fungicides for the control of dollar spot.

#### EFFECTS OF DEW REMOVAL AND TRINEXAPAC-ETHYL ON FUNGICIDE EFFICACY TO CONTROL DOLLAR SPOT ON GOLF COURSE CREEPING BENTGRASS (AGROSTIS STOLONIFERA L.) FAIRWAYS

#### **ABSTRACT**

Dollar spot, caused by the pathogen *Sclerotinia homoeocarpa* F.T. Bennett, is a disease of all turfgrass species and is considered the most economically important disease on golf courses. Many cultural and chemical management practices are necessary to reduce disease symptoms. Canopy moisture (e.g., leaf wetness) plays an important role in the development of dollar spot and routine removal of dew has been shown to reduce disease severity. The effect of canopy moisture on fungicide efficacy at the time of fungicide application, however, is not well understood. In addition to management practices directly related to the reduction of dollar spot, other inputs like the application of plant growth regulators are common to golf courses for maintaining high quality turf. Trinexapac-ethyl (TE) is one of the most common PGRs used on golf courses, but its influence on dollar spot is unclear. The objective of this field study was to elucidate the influence of dew removal methods at the time of fungicide application on dollar spot control within turfgrass regulated by TE. Field studies were initiated at the Joseph Valentine Turfgrass Research Center on a mature 'Penneagle' creeping bentgrass (Agrostis stolonifera L.) fairway. Main factors in the study included three dew removal strategies (untreated, rolled and mowed) prior to the application of fungicides (untreated, chlorothalonil, propiconazole and iprodione). All treatments were applied to turfgrass previously treated with TE treatments (untreated and TE). Dollar spot infection centers (DSIC) and area under the disease progress curve data were analyzed using the PROC MIXED procedure in SAS. The presence or absence of dew at the time of fungicide application had no influence fungicide

efficacy. The effect of regulation by TE on fungicide-treated turf had little influence on dollar spot severity. However, the effect of regulation on non-fungicide treated turf resulted in a significant reduction in DSIC. Based on the results of this study, dew removal prior to the application of fungicides in the morning is an unnecessary step and does not influence fungicide efficacy. Although TE had little influence on disease severity where fungicides were applied, its use in areas where fungicide applications are restricted or too cost prohibitive may provide a small, but beneficial reduction in dollar spot.

#### **INTRODUCTION**

Dollar spot, caused by the pathogen *Sclerotinia homoeocarpa* F.T. Bennett, is a disease of all turfgrass species and is considered the most economically important disease on golf courses. Canopy moisture (e.g., leaf wetness) plays an important role in the development of dollar spot. It is suggested that displacement of dew in the morning can reduce symptoms by interrupting prolonged periods of leaf wetness required for disease development. In addition to the use of cultural practices, fungicides are often applied throughout the season to provide adequate disease suppression.

Mowing practices and dew removal strategies associated with fungicide performance on dollar spot control have been recently evaluated (McDonald et al., 2006; Putman and Kaminski, 2011; Pigati et al., 2010; Delvalle et al., 2011). Putman and Kaminski (2011) found that mowing frequency (e.g. 2, 4, or 6 days week<sup>-1</sup>) had no influence on fungicide performance. However, in the absence of fungicides, less frequently mowed turf (2 day week<sup>-1</sup>) resulted in less dollar spot than turf mown 4 or 6 days week<sup>-1</sup>. The author suggested more frequent mowing may weaken host defenses and alter plant growth habit, and subsequently create an environment more favorable for pathogen growth and infection. In another study, Delvalle et al. (2011) reported that dollar spot control with fungicides can be extended by daily dew removal or increased mowing frequency. Increased mowing frequency likely results in a reduction of dollar spot due to dew removal and/or physical disruption and removal of inoculum (Pigati et al., 2010; Delvalle et al., 2011).

Golf course superintendents often apply pesticides early in the morning due to the concern of potential pesticide exposure and to avoid play. This is the time of the day when a significant amount of dew may be present on the surface. Although numerous research studies

have shown the benefits of routine dew removal, information related to the presence of dew at the time of fungicide application is relatively limited. McDonald et al. (2006) reported no significant differences in dollar spot severity when fungicides were sprayed in the morning with the dew present versus dew displaced. However, they reported that chlorothalonil occasionally provided greater dollar spot control when applied in the AM after dew displacement or at noon to a dry canopy when compared to morning applications to dew-covered turf. It was suggested that improved chlorothalonil performance in the absence of dew may be possible because significant amounts of the fungicide would adhere to the dry foliage and/or would not become diluted. In addition, dew on the plant surface may enhance the pathogen's ability to adhere itself to the leaf surface, which further helps the pathogen to resist displacement by flowing water (Agrios, 2005). In a recent study, Pigati et al. (2010) concluded that morning mowing prior to fungicide applications (chlorothalonil, boscalid, iprodione, and propiconazole) improved the performance of all fungicides compared to plots mowed in the afternoon. Although there is information for dollar spot control related to dew removal strategies alone or following fungicide applications, the impact of dew at the time of fungicide application is still not well understood.

Plant growth regulators (PGRs) have become a conventional golf course turf management practice to regulate turfgrass growth, suppress certain weeds, reduce mowing frequency and clipping yield, and enhance turfgrass color, quality and density (Watschke et al., 1992). Select PGRs have been shown to suppress dollar spot and enhance fungicide efficacy (Burpee et al., 1996). Trinexapac-ethyl (TE), paclobutrazol and flurprimidol are the most commonly used PGRs to suppress turfgrass growth on golf courses in the United States. This group of PGRs regulates plant growth by inhibiting cell elongation in the gibberellic acid biosynthesis pathway (Burpee et al., 1996; Fidanza et al., 2006). Flurprimidol and paclobutrazol

are fungistatic to *S. homoeocarpa* and have been shown to suppress dollar spot when applied alone or in combination with effective dollar spot fungicides (Burpee et al., 1996; Fidanza et al., 2006).

Unlike the fungistatic effects of flurprimidol and paclobutrazol, TE is considered less likely to affect dollar spot severity or fungicide performance (Burpee et al, 1996; Stewart et al., 2007). Several studies, however, have reported that TE improved grass tolerance to abiotic stress and may stimulate non-fungistatic mechanisms that contribute to disease suppression (Burpee et al., 1996; Golembiewski and Danneberger, 1998; Zhang and Schmidt, 2000; McCann and Huang, 2007; Xu and Huang, 2010).

Results from research on the influence of TE on dollar spot severity, however, are inconsistent. Field research findings have described neutral, beneficial or negative effects of TE on fungicide performance for the control of dollar spot (Golembiewski and Danneberger, 1998; Zhang and Schmidt, 2000; Fidanza et al., 2006, Stewart et al., 2008; Putman and Kaminski, 2011). Burpee et al. (1996) found that when TE was applied alone, it had no significant effect on dollar spot. When applied in combination with chlorothalonil, iprodione and propiconazole, however, TE enhanced fungicide efficacy in one year of the two-year study. Kaminski and Putman (2011) reported that TE had no influence on dollar spot when applied in combination with fungicides. In the absence of fungicides, however, TE has been shown to significantly suppress the disease (Golembiewski and Danneberger, 1998; Putman and Kaminski, 2011). In another study conducted by Stewart et al. (2008) on creeping bentgrass, TE rarely influenced dollar spot severity or fungicide performance when applied prior to the onset of dollar spot symptoms. When applied curatively, however, turf recovery from dollar spot damage following applications of chlorothalonil and propiconazole was significantly delayed within TE-treated

plots in some occasions. The authors suggested that diminished turf growth may reduce fungicide uptake and limit the suppressive effect of the active ingredient, thereby delaying recovery from the outbreak.

Limited information is available related to the influence of dew present at the timing of fungicide applications to regulated turf. Therefore, the objectives of this research are to: i) evaluate the residual effectiveness of fungicides from a single preventive application on dollar spot control as influenced by dew removal at the time of application; and ii) determine the effect of turfgrass regulation by TE on residual effectiveness of fungicides for the control of dollar spot.

#### MATERIALS AND METHODS

A two-year field study was conducted at the Joseph Valentine Turfgrass Research Center located in University Park, PA. The site was a nine year-old mixed stand of 'Penneagle' creeping bentgrass (*Agrostis stolonifera* L.) and annual bluegrass (*Poa annua* L.) (90%/10%) maintained similar to a golf course fairway. Soil was a Hagerstown silt loam (fine, mixed, mesic, Typic Hapludalf) with a pH of 6.6, Mehlich-3 P at 224 kg ha<sup>-1</sup>, exchangeable K at 0.16 cmol kg<sup>-1</sup> of soil, and a CEC of 11.1cmol<sub>c</sub> kg<sup>-1</sup> of soil. The area was mowed three times per week with a John Deere triplex 7500 Precision Cut fairway mower (John Deere, Moline, IL) set to a bench height of 1.3 cm. Clippings were collected in baskets and removed from the site. The site was irrigated as needed to prevent wilt. The study was arranged as a randomized complete split-plot design with four replications, and was completed on three separate occasions (Study I, late summer 2011; Study II, spring 2012; and Study III, late summer 2012) in areas adjacent to one another. Whole plots measured 18 m x16 m and subplots measured 0.9 m x1.8 m. Main factors included three dew removal strategies (untreated, rolled and mowed) prior to the application of four fungicides. An additional main factor included the regulation of creeping bentgrass by the plant growth regulator TE.

Fungicide treatments included chlorothalonil (Daconil Ultrex 82.5 WDG, Syngenta Crop Protection, Greensboro, NC) applied at 8.17 kg a.i. ha<sup>-1</sup>, propiconazole [cis-trans-1-(2-(2,4dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl)-1H-1,2,4-trizole] (Banner Maxx, Syngenta Crop Protection, Greensboro, NC) applied at 0.67 kg a.i.ha<sup>-1</sup>, iprodione (3-3,5-dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1 imidazolidinecarboxamide) (Chipco 26 GT, Bayer Environmental Science, Montvale, NJ) applied at 2.21 kg a.i. ha<sup>-1</sup> and an untreated control. Plant growth regulator treatments included trinexapac-ethyl (Primo Maxx, Syngenta Crop Protection, Greensboro, NC) applied at 0.09 kg a.i. L ha<sup>-1</sup> and an untreated control. Creeping bentgrass plots placed under growth regulation were treated with two applications of TE on a 14-day interval prior to fungicide application and dew removal treatment.

Prior to the application of fungicides, dew removal treatments were implemented. Dew removal treatments consisted of mowing the area, running the mower over the area with the reels disengaged or no dew removal. Plots were mowed to a height of 1.3 cm using a John Deere Triplex 7500 Precision Cut fairway mower. Clippings were collected in baskets and removed from the site. For each of the three study evaluations (Study I, II, and III), dew removal and

mowing treatments were initiated between 0700 and 0730 h and all fungicides were sprayed within 30 min.

Fungicide and PGR treatments were applied with a  $CO_2$  pressurized (276 kPa) sprayer equipped with an air-induction flat fan nozzle (AI9504E) calibrated to deliver water at 407 liters ha<sup>-1</sup>. While fungicides and dew removal treatments were applied once for each test, trinexapacethyl applications were continued until the end of each study for a total of five applications to maintain turfgrass regulation. All plots were mowed 1-d prior to dew removal and fungicide application and were mowed again 2-d following treatments. For general maintenance, the site was mowed 3 d wk<sup>-1</sup> based on the regular maintenance schedule as described above.

Study I was conducted in late summer of 2011. The site was fertilized with urea (46-0-0) at a rate of 11 kg N ha<sup>-1</sup> in April 2011. Chlorothalonil was applied to the site at 8.17 kg a.i. ha<sup>-1</sup> on 21 July 2011 to control dollar spot prior the initiation of fungicide treatments. Trinexapacethyl treatments were initiated on 21 July and repeated on 2-wk intervals for a total of five applications. Fungicide and dew removal treatments were applied on 17 Aug.

The second replication of the study was conducted in spring of 2012 (Study II). On 18 April, 2012, the site used for study was fertilized with urea (46-0-0) at a rate of 11 kg N ha<sup>-1</sup>. Dollar spot became active early in spring 2012 and therefore chlorothalonil was applied on two occasions at 8.2 kg a.i. ha<sup>-1</sup> and 4.5 kg a.i. ha<sup>-1</sup> on 17 and 24 May, respectively. Trinexapac-ethyl treatments were initiated on 3 May and repeated every 2 weeks for a total of five applications. Fungicide and dew removal treatments were completed on 31 May.

Study III was initiated in the late summer of 2012. To suppress dollar spot within the study site, chlorothalonil was applied at 12.6 kg a.i. ha<sup>-1</sup> and 4.5 kg a.i. ha<sup>-1</sup> on 8 and 15 August,

respectively. Trinexapac-ethyl treatments were initiated on 23 July and repeated on a 2-wk interval as previously described. Fungicide and dew removal-treatments were initiated on 20 August.

Dew present at the time fungicides and dew removal treatments were applied was quantified by physically blotting the turf canopy with pre-weighed tissue papers (Kleenex, Kimberly-Clark Global Sales. LLC, Neenah, WI). Dew was removed from the leaf surface within a 0.09 m<sup>2</sup> wooden square frame as previously described (Williams et al., 1998; Delvalle et al., 2011). Dew was quantified from six random locations adjacent to each study area, and the tissue papers were weighed immediately to determine the amount of moisture absorbed from the turfgrass canopy.

Trace disease symptoms were present in the study area at the time fungicide and dew treatments were initiated. Once fungicides were applied, dollar spot severity was assessed by visually counting the number of dollar spot infection centers (DSIC) within each plot. Each study was concluded when individual infection centers could no longer be distinguished in the most severely affected plots. Total disease severity was assessed for each study by determining the area under disease progress curve (AUDPC). The AUDPC values were calculated using the formula:  $\sum [(x_i + x_{i+1})/2][t_{i+1} - t_i]$ , where i = 1,2,3...n-1is the number of ratings taken,  $x_i$  is the number of the DSIC at each rating, and the  $t_{i+1} - t_i$  is time between ratings (Madden et al., 2007). Dates used to calculate AUDPC values were 16 August to 22 September in 2011 (Study I) and 30 May to 10 July (Study II) and 21 August to 24 September (Study III) in 2012. All statistical analyses were performed using PROC MIXED of SAS v. 9.3 (SAS institute, Cary, NC). Means were separated at *P*≤0.05 using Tukey's Least Significant Difference test.

#### RESULTS

Dollar spot was allowed to naturally progress and disease pressure was considered moderate to severe during all three evaluations in both years. Trace levels of dollar spot were present at the initiation of each fungicide and dew treatment. Based on the plot of the residuals, all DSIC data and AUDPC values required a square root transformation prior to analyses. A significant study effect was observed for AUDPC data (P = 0.0286) and therefore seasonal dollar spot severity was assessed for each year independently.

Study I. Trace levels of dollar spot ( $\leq$ 3 DSIC) were present when fungicide treatments were initiated on 17 August 2011. A significant fungicide effect was first observed on 23 August and remained significant (*P*< 0.0001) on all remaining rating dates (Table 1). On all dates, no differences in DSIC were observed among fungicides and all fungicides provided significant disease suppression when compared to the untreated control.

As disease pressure increased and the impact of the fungicides began to deteriorate, the main effect of PGR became significant. By 6 September, plots treated with TE had significantly fewer DSIC when compared to the untreated control plots (Table 1). Reductions in DSIC from plots treated with TE were observed on 7 of 13 rating dates and ranged from 13 to 39% fewer infection centers when compared to plots receiving no TE.

A total of 2310 L dew ha<sup>-1</sup> was present when fungicides were applied. The main effect of dew only was significant on a single rating date in Study I (Table 1). On that date (19 September), plots that were initially mowed prior to the application of fungicides had greater DSIC when compared to plots in which dew was not removed. On 9 and 12 September, a significant PGR x fungicide interaction was observed (Table 1). On these two rating dates, no

difference in dollar spot between PGR treatments was observed within fungicide-treated plots. However, TE significantly reduced DSIC by 44 to 49% within plots receiving no fungicide (Table 2).

*Study II.* Dollar spot activity was observed on 4 May, 2012 and recovery applications of chlorothalonil were required prior to initiating fungicide treatments. An average of 5 to 7 DSIC were present when fungicide and dew treatments were initiated on 31 May. Dollar spot was severe during study II and two major peaks in severity were observed during the epidemic (Figure 2).

Similar to Study I, the main effect of fungicide was observed on 6 June (5 days after treatments) and remained significant on all dates through the end of the study (Table 3). Although the greatest level of dollar spot was observed in turf within untreated plots, differences among fungicides were present on select dates. In general, plots treated with iprodione resulted in the fewest DSIC throughout the study.

The main effect of PGR was significant on 9 of 15 rating dates including the last 7 ratings after fungicide performed had waned (Table 3). On dates in which the main effect of PGR was significant, DSIC within plots treated with TE was reduced an average of 20 to 43%.

A significant PGR x fungicide interaction was observed on 9 of 15 rating dates (Table 3). Similar to Study I, differences were observed primarily among PGR treatments within plots receiving no fungicides and no differences among PGR treatments were observed within plots receiving any fungicides (Table 2). For Study II, a total of 978 L of dew ha<sup>-1</sup> was present at the time of fungicide application. The main effect of dew was significant only on 2 rating dates. On both dates (18 and 24 June), plots that were initially mowed prior to the application of fungicides had lower DSIC when compared to plots in which dew was not removed (Table 3).

*Study III*. Trace levels of dollar spot (<1 DSIC) were present when fungicide treatments were initiated on 21 August 2012. In study III, dollar spot developed rapidly for up to 4 weeks after fungicides were applied, but disease pressure naturally subsided later in September (Figure 3). No significant interactions were observed in Study III (Table 4).

The main effect of fungicide was observed on 29 August (8 days after treatments) and remained significant on all rating dates through the end of the study (Table 4). All fungicides reduced dollar spot when compared to the untreated control and few differences were observed among fungicides. Similar to study II, plots treated with iprodione generally had the fewest DSIC.

The main effect of PGR became significant by 5 September (Table 4). Fewer DSIC were observed within plots treated with TE on 8 of 12 rating dates. When compared to the untreated control plots, TE-treated turf had 17 to 35% fewer DSIC.

Dew present at the time fungicides were applied was 1385 L ha<sup>-1</sup>. The main effect of dew, however, was only significant on a single rating date. On 7 September, plots that were initially rolled prior to the application of fungicides had fewer DSIC when compared to plots in which dew was not removed.

Total disease severity for each study was assessed by determining the area under the disease progress curve. Differences among study evaluations existed and therefore AUDPC values for each study are reported individually. No significant interactions were present for AUDPC values in any study (Table 5). Differences among AUDPC values for all main effects and for each study were similar to the results on individual rating dates. In all studies, all fungicides resulted in lower AUDPC values when compared to the untreated control plots and in Study II and III, lower total disease values generally were observed within plots treated with

iprodione (Figures 1 to 3). The impact of TE on dollar spot pressure as a main effect was again significant in all studies.

#### **DISCUSSION**

In this study, fungicides were applied once. The advantage of an experiment that follows disease progress through the effective duration of a single fungicide application is that the bias associated with repeated applications is eliminated (Stewart et al., 2008). Therefore, the possible small effects such as the influence of TE or dew removal methods at the time of fungicide application on fungicide performance can be more accurately determined (Stewart et al., 2008).

It has been suggested that large quantities of dew remaining on the turfgrass canopy may dilute or reduce the amount of fungicide that could adhere to the foliage (McDonald et al., 2006). They reported improved chlorothalonil performance for the control of dollar spot when applied to a dry canopy after 12:00 PM when compared to AM applications. They also occasionally observed better performance in dollar spot control from AM applications made to turf in which the dew was removed when compared to applications made to turf with dew. Based on the results of this study, the presence or absence of dew at the time of fungicide application appears to have little influence on fungicide performance or residual effectiveness. Similar to McDonald et al. (2006), when chlorothalonil was applied to dew displaced turf (rolled or mowed), it generally provided greater control when compared to applications made on dew present turf. However, none of those differences were significant. Slight differences in experimental design, geography, disease pressure, and/or other factors may have been responsible for the slight differences observed in these studies.

In this study, all chemicals were applied at the optimal condition as for fairway height turf, using a CO<sub>2</sub> pressurized (276 kPa) sprayer equipped with an air-induction flat fan nozzle (AI9504E) and calibrated to deliver water at 407 liters ha<sup>-1</sup> (Couch, 1984; McDonald et al., 2006; Kaminski and Fidanza, 2009). Optimal application strategies may minimize the influence of the presence of dew. The impact of dew on fungicide efficacy when applying fungicides under varying conditions (e.g., higher or lower water volumes) remains unknown. There are also many additional considerations to take into account when deciding if dew removal is necessary. For instance, in our study, the concentration of chlorothalonil within dew droplets was observed when the fungicide was applied to turf in which dew was present. Additionally, drying times of the fungicides were notably shorter when applied to turf in which the dew was removed when compared to applications to dew-laden turf. When taking into consideration other activities on a golf course (e.g., golfers), the removal of dew prior to the application of a fungicide may assist in a more rapid drying of the product and therefore reduce the risk of displacement prior to drying and/or exposure to the golfers.

Fungicides effectively reduced dollar spot throughout the study when compared to turf not receiving fungicide applications. Although few differences among fungicides existed, plots treated with iprodione generally provided equal or greater dollar spot suppression when compared to chlorothalonil or propiconazole. As a contact fungicide, the residual control of the fungicide chlorothalonil is expected to be relatively short (<14 days). Slight differences in dollar spot control among propiconazole and iprodione is expected. Latin (2006) suggested that it is likely the chemotherapeutic properties of penetrant fungicides may also account for the discrepancies among fungicides field performance. Additionally, the study site has a known history of reduced sensitivity to propiconazole.

Although the reported results of TE on dollar spot has been limited and conflicting, results from this work indicate that repeated applications of TE can result in a reduction of dollar spot. These reductions, however, generally do not become apparent until fungicide efficacy begins to wane. In all three studies reported here, differences in dollar spot were observed approximately 15 to 20 days after fungicide treatments were initiated. Although the general trend was lower DSIC in all fungicide-treated plots (Table 2; Figure 4), significant reductions from TE only were observed within plots receiving no fungicide (Table 2). These results are similar to those described previously in which the impact of TE was more prominent on non-fungicide treated turfgrass (Golembiewski and Danneberger, 1998; Putman and Kaminski, 2011). In most cases TE has been shown to have little or no impact on fungicide efficacy (Fidanza et al., 2006; Stewart et al., 2008; Putman and Kaminski, 2011).

In contrast to our findings, Burpee et al. (1996) reported a significant efficacy enhancement of chlorothalonil, iprodione and propiconazole when applied in combination with TE. When applied alone, TE did not affect dollar spot. Based on previous findings that TE is not fungistatic to *S. homoeocarpa*, Burpee suggested that TE-enhancement of fungicides may have been due to a reduction in the amount of leaf tissue containing fungicides removed by mowing (Burpee et al., 1996). In a Connecticut field study, although applications of TE resulted in a reduction in clipping yield, removal of protected tissue by mowing did not appear to influence the fungicide residual efficacy (Putman and Kaminski, 2011). Effective concentrations of various fungicides, however, are believed to remain in the turf canopy for only 1 to 2 weeks based on the efficacy half-life (Latin, 2006).

Others have suggested that the physiological response resulting from applications of TE may strengthen the grass or activate natural defense mechanisms (Zhang and Schmidt, 2000;

McCann and Huang, 2007; Xu and Huang, 2010). Zhang and Schmidt (2000) found that TE increased superoxide dismutase (SOD) activity and influenced photochemical activity and chlorophyll content of creeping bentgrass. They also suggested that increased SOD activity may be responsible for observed increases in creeping bentgrass tolerance to both drought stress and dollar spot. These indirect benefits may be influential in TE's ability to reduce dollar spot. Although the application of TE reduced dollar spot in this study, the suppression was limited and the agronomic benefits small. Applications of TE did not improve or extend fungicide efficacy, nor result in a commercially acceptable reduction in dollar spot for golf course turf.

Results of this study indicate that dew removal prior to a morning fungicide application is not advantageous. Additionally, although the benefits of TE were observed after fungicide performance began to deteriorate; the reductions were limited and would likely not be considered acceptable to most turfgrass managers. However, in situations where fungicide use is limited or restricted the slight reductions in dollar spot from repeated applications of TE may prove helpful.

	Dollar spot severity <sup><math>\dagger</math></sup>												
	16 Aug	20 Aug	23 Aug	26 Aug	30 Aug	02 Sept	06 Sep	09 Sep	12 Sep	16 Sep	19 Sep	22 Sep	26 Sep
Treatment <sup>‡</sup>						Infection	centers pl	lot <sup>-1</sup>					
Dew													
Untreated	2 a <sup>§</sup>	4 a	4 a	3 a	5 a	4 a	7 a	21 a	20 a	27 a	29 b	39 a	80 a
Rolled	3 a	8 a	6 a	6 a	9 a	8 a	11 a	28 a	24 a	34 a	34ab	43 a	87 a
Mowed	3 a	7 a	6 a	6 a	7 a	6 a	11 a	25 a	23 a	31 a	39 a	40 a	88 a
PGR													
Untreated	3 a	7 a	6 a	6 a	7 a	7 a	12 a	32 a	28 a	38 a	41 a	49 a	93 a
Trinexapac-ethyl	2 b	6 a	5 a	5 a	6 a	5 a	8 b	28 b	17 b	23 b	26 b	32 b	76 b
Fungicides													
Untreated	3 a	8 a	11 a	16 a	21 a	21 a	27 a	58 a	52 a	64 a	63 a	71 a	122 a
Chlorothalonil	3 a	5 a	3 b	2 b	3 b	2 b	6 b	17 b	16 b	23 b	29 b	32 b	73 b
Propiconazole	2 a	6 а	4 b	2 b	3 b	1 b	4 b	12 b	11 b	19 b	22 b	30 b	70 b
Iprodione	3 a	6 a	3 b	1 b	1 b	0 b	2 b	12 b	11 b	17 b	21 b	29 b	74 b
Source of variation													
Dew	0.2723	0.1598	0.2275	0.5380	0.2012	0.2018	0.0574	0.0898	0.3073	0.1957	0.0372	0.7446	0.2559
PGR	0.0327	0.5119	0.7314	0.9461	0.9438	0.3150	0.0062	<.0001	<.0001	<.0001	<.0001	<.0001	0.0004
Fungicide	0.5357	0.7763	0.0010	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Dew*Fungicide	0.1645	0.3964	0.3147	0.4655	0.2221	0.5553	0.7157	0.4844	0.0517	0.0633	0.1258	0.1150	0.6089
PGR*Fungicide	0.8414	0.8139	0.5641	0.7467	0.7468	0.6918	0.3148	0.0074	0.0074	0.0690	0.5858	0.0563	0.6491
Dew*PGR*Fung	0.8294	0.9837	0.8786	0.9634	0.8278	0.9132	0.9737	0.8152	0.5083	0.8751	0.9411	0.9345	0.8633

Table 1. Influence of dew removal methods, plant growth regulator (PGR) and fungicides on dollar spot severity on a creeping bentgrass research fairway, late summer 2011 (study I).

Dollar spot severity was assessed by counting the number of infection centers per plot. All data were square root transformed prior to analyses, but actual Ť means are shown.

Trinexapac-ethyl was applied on 21 Jul; 4, 17, and 30 Aug; 14 Sep. All fungicide and dew removal treatments were initiated on 17 Aug. <sup>8</sup> Means followed by the same letter are not significantly different at  $P \le 0.05$  according to the Tukey's Least Significant Difference test.

•	Dollar spot severity <sup>†</sup>										
					Infectio	n centers	plot <sup>-1</sup>				
Treatment <sup>‡</sup>	Stu	Study I Study II									
PGR <sup>‡</sup>	09 Sep	12 Sep	06 Jun	08 Jun	13 Jun	15 Jun	18 Jun	21 Jun	24 Jun	26 Jun	29 Jun
Chlorothalonil											
None	22 c <sup>§</sup>	22 bc	4 ab	4 bc	2 cde	5 c	12 b	23 bc	34 bc	28 b	18 bc
Trinexapac-ethyl	11 cd	10 cd	3 b	4 bc	2 cde	4 c	10 bc	16 bcd	27 bcd	17 bc	11 cd
Propiconazole											
None	13 cd	13 cd	4 ab	6 bc	4 bc	6 c	7 bc	12 cd	21 cd	21 bc	13 cd
Trinexapac-ethyl	11 d	10 d	4 ab	6 b	3 bcd	3 c	4 bc	8 d	11 d	8 c	4 d
Iprodione											
None	14 cd	12 cd	2 b	1 c	1 e	1 c	2 c	5 d	11 d	9 c	5 d
Trinexapac-ethyl	10 d	10 d	3 ab	3 bc	1de	2 c	5 bc	8 cd	14 d	9 c	6 cd
Untreated											
None	77 a	66 a	6 a	15 a	17 a	36 a	45 a	61 a	79 a	66 a	49 a
Trinexapac-ethyl	39 b	37 b	3 ab	6 b	8 b	21 b	30 a	37 b	45 b	35 b	30 b

Table 2. Fungicide x plant growth regulator interactions on dollar spot severity on a creeping bentgrass research fairway, Study I and Study II.

Dollar spot severity was assessed by counting the number of infection centers per plot. All data were square root transformed prior to analyses, but actual means are shown.

<sup>‡</sup> Trinexapac-ethyl was applied on 21 Jul; 4, 17, and 30 Aug; 14 Sep in 2011(Study I), and on 3, 17, and 31 May; 14 and 28 Jun in 2012 (Study II). All fungicide and dew removal treatments were initiated on17 Aug in 2011 and 31 May in 2012.

<sup>§</sup> Means followed by the same letter are not significantly different at  $P \le 0.05$  according to the Tukey's Least Significant Difference test.

	Dollar spot severity <sup>†</sup>									
-	30 May	04 Jun	06 Jun	08 Jun	11 Jun	13 Jun	15 Jun	18 Jun		
Treatment <sup>‡</sup>	Infection centers plot <sup>-1</sup>									
Dew										
Untreated	7 a <sup>§</sup>	4 a	3 a	6 a	5 a	6 a	12 a	18 a		
Rolled	5 a	3 a	4 a	6 a	5 a	5 a	10 a	14 ab		
Mowed	5 a	3 a	3 a	5 a	5 a	4 a	7 a	11 b		
PGR										
Untreated	6 a	4 a	4 a	7 a	6 a	6 a	12 a	16 a		
Trinexapac-ethyl	5 a	3 b	3 a	5 a	4 a	4 b	7a	12 a		
Fungicides										
Untreated	6 a	4 a	5 a	11 a	13 a	12 a	29 a	37 a		
Chlorothalonil	5 a	4 a	3 b	4 bc	3 bc	2 c	4 b	11 b		
Propiconazole	6 a	3 a	4 ab	6 b	4 b	4 b	4 b	6 bc		
Iprodione	6 a	3 a	2 b	2 c	1c	1c	1 b	3c		
Source of variation										
Dew	0.1597	0.2934	0.4003	0.8897	0.9341	0.1440	0.0543	0.0396		
PGR	0.4638	0.0116	0.6938	0.8629	0.5014	0.0089	0.0777	0.2969		
Fungicide	0.9698	0.8338	0.0105	<.0001	<.0001	<.0001	<.0001	<.0001		
Dew*Fungicide	0.8134	0.7447	0.3809	0.3932	0.8522	0.5687	0.4527	0.7783		
PGR*Fungicide	0.2915	0.0857	0.0488	0.0035	0.0693	0.0063	0.0205	0.0156		
Dew*PGR*Fung	0.6346	0.6826	0.8605	0.9109	0.1517	0.6710	0.6614	0.5063		

Table 3. Influence of dew removal methods, plant growth regulator (PGR) and fungicides on dollar spot severity on a creeping bentgrass research fairway, spring 2012 (study II).

<sup>†</sup> Dollar spot severity was assessed by counting the number of infection centers per plot. All data were square root transformed prior to analyses, but actual means are shown.

<sup>\*</sup> Trinexapac-ethyl was applied on 3, 17, and 31 May; 14 and 28 Jun. All fungicides and dew removal treatments were initiated on 31 May. <sup>§</sup> Means followed by the same letter are not significantly different at  $P \le 0.05$  according to the Tukey's Least Significant Difference test.

	Dollar spot severity <sup>†</sup>									
	21 Jun	24 Jun	26 Jun	29 Jun	03 Jul	06 Jul	10 Jul			
Treatment <sup>‡</sup>			]	infection cente	ers plot <sup>-1</sup>					
Dew										
Untreated	25 a <sup>§</sup>	36 a	29 a	20 a	20 a	26 a	69 a			
Rolled	22 a	28 ab	23 a	16 a	17 a	24 a	99 a			
Mowed	18 a	27 b	21 a	16 a	17 a	35 a	86 a			
PGR										
Untreated	25 a	36 a	31 a	21 a	21 a	35 a	98 a			
Trinexapac-ethyl	18 b	24 b	17 b	13 b	14 b	22 b	78 b			
Fungicides										
Untreated	49 a	62 a	50 a	40 a	30 a	41 a	97 ab			
Chlorothalonil	19 b	30 b	23 b	15 b	20 a	37 a	102 a			
Propiconazole	10 c	16 c	14 bc	9 bc	12 b	20 b	82 ab			
Iprodione	7 c	13 c	9 c	5 c	9 b	16 b	71 b			
Source of variation										
Dew	0.1114	0.0408	0.0638	0.2362	0.5340	0.0666	0.8593			
PGR	0.0295	0.0032	<.0001	0.0025	0.0018	0.0033	0.0035			
Fungicide	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0086			
Dew*Fungicide	0.9682	0.7448	0.2956	0.3786	0.4413	0.2990	0.7069			
PGR*Fungicide	0.0141	0.0212	0.0407	0.0327	0.1586	0.1663	0.8386			
Dew*PGR*Fung	0.2964	0.4767	0.5369	0.4749	0.4964	0.6833	0.0888			

Table 3 (con't). Influence of dew removal methods, plant growth regulator (PGR) and fungicides on dollar spot severity on a creeping bentgrass research fairway, spring 2012 (study II).

<sup>†</sup> Dollar spot severity was assessed by counting the number of infection centers per plot. All data were square root transformed prior to analyses, but actual means are shown.

<sup>‡</sup> Trinexapac-ethyl was applied on 3, 17, and 31 May; 14 and 28 Jun. All fungicides and dew removal treatments were initiated on 31 May. <sup>§</sup> Means followed by the same letter are not significantly different at  $P \le 0.05$  according to the Tukey's Least Significant Difference test.

	Dollar spot severity <sup>†</sup>											
	21 Aug	27 Aug	29 Aug	02 Sep	05 Sep	07 Sep	09 Sep	12 Sep	14 Sep	17 Sep	21 Sep	24 Sep
Treatment <sup>‡</sup>	Infection centers plot <sup>-1</sup>											
Dew												
Untreated	1 a <sup>§</sup>	0 a	5 a	12 a	26 a	38 a	36 a	49 a	54 a	46 a	43 a	41 a
Rolled	1 a	1 a	4 a	7 a	23 a	25 b	29 a	41 a	44 a	37 a	37 a	33 a
Mowed	1 a	1 a	5 a	11 a	20 a	27ab	29 a	42 a	50 a	37 a	40 a	39 a
PGR												
Untreated	1 a	1 a	6 a	12 a	27 a	34 a	36 a	49 a	54 a	45 a	44 a	42 a
Trinexapac-ethyl	1 a	1 a	3 a	9 a	19 b	26 b	27 b	39 b	45 b	35 b	36 b	33 b
Fungicides												
Untreated	1 a	1 a	17 a	37 a	69 a	70 a	73 a	81 a	90 a	77 a	79 a	68 a
Chlorothalonil	1 a	0 a	0 b	1 b	9 b	14 c	15 b	31 b	36 b	28 b	28 b	28 b
Propiconazole	1 a	0 a	0 b	3 b	8 b	23 b	24 b	37 b	42 b	35 b	34 b	35 b
Iprodione	1 a	0 a	0 b	0 b	7 b	13 c	14 b	27 b	29 c	20 c	19 c	20 c
Source of variation												
Dew	0.4783	0.9743	0.7116	0.2976	0.1784	0.0385	0.2864	0.5071	0.3397	0.2805	0.6120	0.1687
PGR	0.2265	0.4552	0.0853	0.1679	0.0010	0.0059	0.0041	0.0213	0.0282	0.0112	0.0286	0.0116
Fungicide	0.8235	0.1256	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Dew*Fungicide	0.6645	0.5146	0.9650	0.5284	0.6470	0.6201	0.2920	0.2893	0.1737	0.6406	0.1787	0.2703
PGR*Fungicide	0.7428	0.7061	0.1541	0.8340	0.7775	0.3282	0.5073	0.8243	0.5504	0.6668	0.4748	0.3182
Dew*PGR*Fung	0.5741	0.1909	0.9600	0.6690	0.8310	0.9445	0.6090	0.9520	0.7137	0.5508	0.9824	0.9730

Table 4. Influence of dew removal methods, plant growth regulator (PGR) and fungicides on dollar spot severity on a creeping bentgrass research fairway, late summer 2012 (study III).

<sup>†</sup> Dollar spot severity was assessed by counting the number of infection centers per plot. All data were square root transformed prior to analyses, but actual means are shown.

Trinexapac-ethyl was applied on 23 Jul; 6 and 21 Aug; 4 and 17 Sep. All fungicide and dew removal treatments were initiated on 21 Aug. <sup>8</sup> Means followed by the same letter are not significantly different at  $P \le 0.05$  according to the Tukey's Least Significant Difference test.

	Disease severity									
—	Study I	Study II	Study III							
Treatment <sup>‡</sup>	AUDPC									
Dew										
Untreated	700 a <sup>§</sup>	812 a	925 a							
Rolled	873 a	701 a	753 a							
Mowed	840 a	710 a	781 a							
PGR										
Untreated	959 a	877 a	921 a							
Trinexapac-ethyl	650 b	605 b	719 b							
Fungicides										
Untreated	1598 a	1323 a	1758 a							
Chlorothalonil	602 b	748 b	496 b							
Propiconazole	520 b	511 bc	635 b							
Iprodione	496 b	381 c	391 c							
Source of variation										
Dew	0.1298	0.4574	0.3686							
PGR	<.0001	0.0012	0.0077							
Fungicide	<.0001	<.0001	<.0001							
Dew*Fungicide	0.0950	0.5813	0.3594							
PGR*Fungicide	0.0602	0.0768	0.6970							
Dew*PGR*Fung	0.9674	0.5151	0.8618							

Table 5. Overall dollar spot disease severity as determined by the area under the disease progress curve (AUDPC) on a creeping bentgrass research fairway as influenced by dew removal methods, plant growth regulator and various fungicides.

<sup>†</sup> Overall dollar spot severity was determined using calculated AUDPC (area under disease progress curve) value. All data were square root transformed prior to analyses, but actual means are shown.

<sup>\*</sup> Trinexapac-ethyl was applied on 21 Jul; 4, 17, and 30 Aug; 14 Sep in 2011 (Study I); on 3, 17, and 31 May; 14 and 28 Jun in 2012 (Study II); and on 23 Jul; 6 and 21 Aug; 4 and 17 Sep in 2012 (Study III). All fungicide and dew removal treatments were initiated on17 Aug in Study I, 31 May in Study II and 21 Aug in Study III.

<sup>§</sup> Means followed by the same letter are not significantly different at  $P \le 0.05$  according to the Tukey's Least Significant Difference test.



Figure 1. Dollar spot severity as influenced by the main effect of fungicide (A), plant growth regulator (B) and dew removal technique (C) on a creeping bentgrass research fairway during the late summer of 2011 (Study I). Seasonal dollar spot severity (e.g., area under the disease progress curve) for each treatment is shown next to the last rating date. Individual rating date with significant fungicide x plant growth regulator interactions was labeled with <sup>\*\*</sup>. For each main effect, means with similar letters are not significantly different at  $P \le 0.05$  according to Tukey's Least Significant Difference test.



Figure 2. Dollar spot severity as influenced by the main effect of fungicide (A), plant growth regulator (B) and dew removal technique (C) on a creeping bentgrass research fairway during the late summer of 2011 (Study I). Seasonal dollar spot severity (e.g., area under the disease progress curve) for each treatment is shown next to the last rating date. Individual rating date with significant fungicide x plant growth regulator interactions was labeled with <sup>\*\*</sup>. For each main effect, means with similar letters are not significantly different at  $P \le 0.05$  according to Tukey's Least Significant Difference test.



Figure 3. Dollar spot severity as influenced by the main effect of fungicide (A), plant growth regulator (B) and dew removal technique (C) on a creeping bentgrass research fairway during the late summer of 2011 (Study I). Seasonal dollar spot severity (e.g., area under the disease progress curve) for each treatment is shown next to the last rating date. For each main effect, means with similar letters are not significantly different at  $P \le 0.05$  according to Tukey's Least Significant Difference test.



Figure 4. Dollar spot severity as influenced by the main effect of fungicide (A), plant growth regulator (B) and dew removal techniques (C) on a creeping bentgrass research fairway in Study I (A1-C1), Study II (A2-C2) and Study III (A3-C3). Seasonal dollar spot severity (e.g., area under the disease progress curve) for each treatment is shown next to the last rating date. Data represent only turf treated with a fungicide and do not include data from non-fungicide treated plots. For each main effect, means with similar letters are not significantly different at  $P \le 0.05$  according to Tukey's Least Significant Difference test.

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# APPENDIX: SEASONAL SURVIVAL OF *SCLEROTINIA HOMOEOCARPA* ON VARIOUS PLANT TISSUES

*Sclerotinia homoeocarpa* primarily infects turfgrass foliage. It does not infect roots or stolons directly, but certain mycotoxins have been shown to develop, causing necrosis of creeping bentgrass roots and the apical meristem (Malca and Endo, 1965; Smiley et al., 2005). The pathogen does not form sclerotia, but instead produces darkly pigmented stromata. *S. homoeocarpa* may be capable of surviving in plant debris as a facultative saprophyte for brief periods (Vargas, 2005). The pathogen's overwintering strategy is considered to form darkly pigmented stromata on margin of dollar spot lesions from previous epidemic or survive as dormant mycelium in infected grass tissues and crowns (Couch, 1995; Smiley et al., 2005; Fenstermacher, 1980). Harman, et al., (1998) stated the basic biology of *Sclerotinia homoeocarpa*, including the form in which it exists when it is not causing disease, is not known. He also proposed there are at two stages of the pathogen life cycle: slow-growing quiescent phase and a pathogenic aggressive phase. Detection of the quiescent phase in site where the pathogen killed turf, however, was unsuccessful (Harman, et al., 1998). The form and places that this pathogen overwinter, is still remain unsolved.

A study was designed to further investigate the biology of *S. homoeocarpa* and evaluate its ability to survive within or on various bentgrass tissues at various times of the year. Dollar spot samples were collected monthly from the Joseph Valentine Turfgrass Research Center located in University Park, PA. The site was a mix stand of 'L-93' creeping bentgrass (*Agrostis stolonifera* L.)and annual bluegrass (*Poa annual* L.)(80%/20%) maintained similar to a golf course putting green with known historic occurrences of dollar spot. Soil texture was previously defined as sandy, with a starting pH of 7.2, 1.8% organic matter, Mehlich-3 P at 218 kg ha<sup>-1</sup>, exchangeable K at 0.16 cmol kg<sup>-1</sup> of soil and a cation exchange capacity of 15.3 cmol<sub>c</sub> kg<sup>-1</sup>. The

area was mowed 6 times per week to a height of 3.2 mm. The site was irrigated as needed to prevent wilt. No fungicides were applied during the duration of the experiment.

The survival of S. homoeocarpa within or on plants creeping bentgrass was assessed by isolating the pathogen from various tissues (green leaves, diseased leaves, sheath, crown, stolon and root) on a monthly basis throughout the year (December 2011 to October 2012). For each assessment, three cores (3 cm in diameter) with symptomatic tissues were removed from the site and immediately taken to the lab for isolation. Samples with no visible dollar spot symptoms adjacent to sampled symptomatic tissue were also collected for isolations which served as the asymptomatic control. Plant tissues were sectioned into 3- to 6-mm-long pieces, surface disinfested in 10% sodium hypochlorite for 60 s, and washed three times for 30 s in sterilized water. Tissue was then blotted dry on sterile filter paper prior to plating on antibiotic water agar (AWA) (Bacteriological agar 20 g  $L^{-1}$  with Penicillin G 0.5 g  $L^{-1}$  and streptomycin sulfate 0.5 g  $L^{-1}$ ). Tissues were maintained on AWA for 3 to 5 days before transferring visibly growing mycelia to potato dextrose agar (PDA) (Potato dextrose agar 39 g L<sup>-1</sup>). Positive identification of S. homoeocarpa was based on colony morphology typical for the pathogen after 7 to 19 days of growth on PDA. A total of 10 tissue sections of each leaf type were examined in each monthly assessment. Data were reported as a percentage of successful S. homoeocarpa isolations.

Successful cultures of *S. homoeocarpa* were isolated from symptomatic plants throughout the year, with the exception of March and April. Survive of *S. homoeocarpa* was mostly from leaves with dollar spot lesions. Isolation of *S. homoeocarpa* from green leaves without lesions was rarely successful. Active *S. homoeocarpa* was found on sheath occasionally. Similar to previous findings, *S. homoeocarpa* does not appear to infect crown, stolon or roots (Malca and Endo, 1965; Smiley et al., 2005). *S. homoeocarpa* from any tissue of asymptomatic plants

adjacent to infected turf was not possible. These results suggest that *S. homoeocarpa* is only present within leaves where lesions have already developed and the pathogen does not move readily to all plant parts.

During the winter, samples were obtained from the frozen soil under snow. *S. homoeocarpa* was detected as long as there were green leaves with lesions. In March and April, when the sward appeared dormant, successful isolation of *S. homoeocarpa* was not possible. This is similar to the conclusion from Harman et al. (1999), who stated that it is unable to detect the quiescent phase in site where the pathogen killed turf. It is assumed that during this stage, *S. homoeocarpa* is behaving as a saprophyte. When attempts to isolate on artificial media, other saprophytes may be more aggressive than *S. homoeocarpa* and therefore it was not possible to select for the dollar spot pathogen. Therefore, detection of *S. homoeocarpa* in this saprophyte stage cannot be well achieved by isolation techniques. Genetic detection based on the ITS region may render a promising approach for investigation of *S. homoeocarpa* at this stage.

	Percentage successful S. homoeocarpa isolations <sup>†</sup>											
-	Dec	Nov	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Plant tissues <sup>‡</sup>						(	%					
Symptomatic plants												
Leaf with lesion	70	40	50	30	0	0	50	60	50	80	70	50
Green leaf	20	0	0	0	0	0	0	10	0	0	0	0
Sheath	10	10	20	10	0	0	20	30	0	0	0	20
Crown	0	0	0	0	0	0	0	0	0	0	0	0
Stolon	0	0	0	0	0	0	0	0	0	0	0	0
Root	0	0	0	0	0	0	0	0	10	0	0	0
Asymptomatic plants												
Green leaf	0	0	0	0	0	0	0	0	0	0	0	0
Sheath	0	0	0	0	0	0	0	0	0	0	0	0
Crown	0	0	0	0	0	0	0	0	0	0	0	0
Stolon	0	0	0	0	0	0	0	0	0	0	0	0
Root	0	0	0	0	0	0	0	0	0	0	0	0

Table 6. Percent successful isolation of *Sclerotinia homoeocarpa* from various plant tissues of symptomatic and asymptomatic creeping bentgrass, 2011 to 2012.

<sup>†</sup> Percentage of *S. homoeocarpa* isolations were determined by the number of successful isolations from a total of ten samples of each tissue type.