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VARIATION IN CLARIREEDIA SPP. INHIBITION AND TURFGRASS PHYTOTOXICITY AMONG DEMETHYLATION INHIBITOR FUNGICIDES

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Maureen M. Kahiu

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The thesis of Maureen M. Kahiu was reviewed and approved by the following:

John E. Kaminski
Professor of Turfgrass Management
Thesis Advisor

Pete J. Landschoot
Professor of Agronomy
Director of Graduate Studies

Wakar Uddin
Professor of Plant Pathology

Erin L. Connolly
Professor and Head of Plant Science

ABSTRACT

Dollar spot is arguably the most common and economically important disease in turfgrass systems. Although several cultural practices can be implemented to reduce disease severity, fungicides are often required to meet acceptable standards for golf course turf. Among fungicides available for control of dollar spot, demethylation inhibitor fungicides (DMIs) are one of the most commonly used fungicide group to manage the disease. DMI fungicides have however been reported to cause foliar turfgrass injury when applied in the summer. The objectives of this study were to 1) elucidate the phytotoxicity of commercially available DMIs when applied to annual bluegrass (*Poa annua*) (ABG) and creeping bentgrass (*Agrostis stolonifera*) (CBG) fairways; and 2) determine EC_{50}, relative mean growth, and discriminatory dose values for nine commercially available DMIs for *Clarireedia jacksonii* isolates with varying levels of insensitivity. Fungicide treatments were applied to creeping bentgrass and annual bluegrass fairways for two consecutive years. Triticonazole was found to cause significant injury to annual bluegrass but no significant injury was observed on creeping bentgrass. Mefentrifluconazole had the lowest injury compared to all other treatments on both grass species. Propiconazole caused darkening and thickening of foliage on both annual bluegrass and creeping bentgrass while triadimefon caused injury on creeping bentgrass but not on annual bluegrass. Generally, injury to both turfgrass species started to decline after the third application and completely waned approximately 2 weeks after the fifth and final application. Repeated use of DMI fungicides has previously been reported to cause reduced sensitivity to *Clarireedia* spp. populations and injury to both ABG and CBG. Fifty isolates were screened across nine commercially available DMIs and discriminatory doses for each determined. Isolates of varying sensitivity levels to propiconazole were screened across determined discriminatory doses. In both experiments, mefentrifluconazole, myclobutanil, and triticonazole resulted in the least mycelial growth across sensitive isolates while prothioconazole and triadimefon resulted in the highest radial mycelial growth. On fungicide amended media, relative mean growth was lowest in the sensitive isolates followed by the moderately and highly insensitive isolates respectively.

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CHAPTER I - LITERATURE REVIEW

Introduction

Dollar spot, caused by one of five *Clarireedia* spp., is one of the most common turfgrass diseases of both warm-season and cool-season grasses (Couch, 1985; Salgado-Salazar et al., 2018; Hu et al., 2019). It is arguably one of the most economically important diseases as it may cause considerable damage to turfgrass when prevention or control is untimely. Golf course turfgrass managers spend a large portion of their budget to manage dollar spot (Vargas, 1994).

Symptoms of dollar spot initially appear as circular ‘silver dollar’-sized lesions on closely mowed turf (F.T. Bennett, 1937; Monteith, 1927; Monteith and Dahl, 1932; Smiley et al., 2005). On individual leaf blades of cool-season grasses, lesions are bleached and hourglass in shape with a dark-brown border. Symptoms on warm-season grasses often exhibit oval-shaped lesions with a similar shaped brown border (Smiley et al., 2005). The disease may result in a severe reduction in quality of young and mature turfgrass stands. In the field, turf managers can identify the pathogen by the cottony mycelium that may be present during the morning hours (Smiley et al., 2005). The pathogen can infect the grass through natural openings in the foliage (e.g., stomates) or through direct penetration of the leaves (Smiley et al., 2005). If left untreated, spots may coalesce into large patches that can reduce the uniformity, aesthetics, and playability of highly maintained turf surfaces. Open bare spots left by the disease sometimes harbor weeds as opportunistic pests (Smith et al., 1989; Vargas, 2005).

Taxonomy and biology

For many years, dollar spot was believed to be caused by the ascomycete *Sclerotinia homoeocarpa* F.T. Bennett, but has since been found to be wrongly classified under the genus

Sclerotinia and family Rutstromiaceae (Bennett, 1937). In 2018, the pathogen was reclassified into one of five species within the new genus *Clarireedia* (F.T. Bennett) L.A. Beirn, B.B. Clarke, C. Salgado, and J.A. Crouch (Salgado-Salazar et al., 2018; Hu et al., 2019). Differences among species were based on geography and affected turfgrass type. The initial named species, *C. homoeocarpa,* was found to be the causal agent in the United Kingdom and mainland Europe together with *C. Benetti*. These two isolates represented a minority of the disease-causing isolates identified from a worldwide isolate collection, making up about 29% of the isolates from the collection. Two additional species more commonly observed in the United States and other parts of the world included *C. monteithiana* and *C. jacksonii,* which were found to predominantly be limited to warm and cool-season grasses, respectively (Salgado-Salazar et al., 2018). Differences among the species were distinguished using molecular phylogenic analyses due to lack of distinct morphological characteristics and reproductive structures in the three newly discovered species. Only the type species, *C. homoeocarpa,* and *C jacksonii* produced apothecia when grown *in vitro* (Salgado-Salazar et al., 2018). A fifth species, primarily found in the turfgrass genus *Paspalum* in China was identified and characterized as *Clarireedia paspali* (Hu et al., 2019).

In the Northern United States, most grasses grown on golf courses are susceptible to *Clarireedia* spp., but infection rates are different among species (Chakraborty et al., 2006). Annual bluegrass *(Poa annua* L.*)* and creeping bentgrass (*Agrostis stolonifera* L.*)* are two commonly used and susceptible species. During optimum environmental conditions, mycelia in contact with the foliage produces an appressorium that directly penetrates plant cells (Monteith and Dahl, 1932). The pathogen has also been known to produce a mycotoxin that causes root damage (Endo, 1963).

Epidemiology

Fungi within the *Clarireedia* spp. typically survive as stroma or mycelia in infected plant debris. The pathogen may become active at temperatures as low as 10^{o}C, but disease symptoms generally appear at temperatures ranging from 15 to 30^{o}C and becomes severe during periods of warm days and cool nights (Couch, 1995; Smiley et al., 2005). Prolonged leaf wetness combined with of >85% relative humidity and optimum temperature increases the possibility of disease occurrence (Walsh et al. 1999). The pathogen has also been reported to be more abundant in thatch than in the soil on a golf course fairway (Allan-Perkins et al., 2018).

Dollar spot generally occurs across multiple seasons depending on geographic location and climatic conditions; however, the disease is generally more severe in the spring and autumn (Powell and Vargas, 2001). In temperate climatic regions, it occurs as two seasonal epidemics with the first one in the May to July period and a second one from mid-August to October (Powell and Vargas, 2001). The disease is more severe in dry soils, but also requires periods of prolonged leaf wetness (Smith et al., 1989; Smiley et al., 2005). Disease occurrence is influenced by irrigation and morning mowing. After fungicide treatment applications, plots irrigated 30 min after, were found to have more dollar spot occurrence than those left without irrigation, while morning mowing prior to fungicide application was found to improve dollar spot control (Pigati et al., 2010). Spatial structure of disease incidence in North America did not change over annual epidemics, indicating that dollar spot epidemics were not consistent with polycyclic diseases (Horvath et al., 2007).

Disease forecasting systems have been developed and used previously. A model to predict fungicide timing, based on relative humidity and air temperature, was first developed in 1982 (Mills and Rothwell, 1982) resulting in fungicide applications at relative humidity levels above

90% and air temperature above 25^{o}C. Due to variability in weather conditions amongst different geographical regions, disease warning systems are sometimes erratic. The Smith-Kerns model is a logistics-based dollar spot disease warning system that uses a moving average of relative humidity and average daily air temperatures collected over a period of five days to predict the probability of disease occurrence (Smith et al., 2018).

Dollar Spot Management

Plant nutrition

Inadequate nutrition and irrigation cause stress in the development and survival of turfgrasses. Although the mechanism remains unclear, nitrogen fertility has been found to reduce dollar spot severity (Williams et al., 1996). This could be due to an indirect positive effect of better turfgrass health arising from the improvement of fertility. Poor nitrogen nutrition is associated with reduced plant vigor which may increase susceptibility to infection. Nitrogen availability is positively correlated with turfgrass quality and negatively correlated to dollar spot severity (Freeman, 1969; Harman, 1991; Melvin and Vargas, 1994; Landschoot and McNitt, 1997).

Nitrogen source, however, has not been shown to influence dollar spot severity on creeping bentgrass fairways. Studies involving organic fertilizers have yielded inconsistent results. Studies have shown that Milorganite, a sewage sludge nutrient source, significantly reduced the severity of dollar spot (Markland et al.,1969; Landschoot and McNitt, 1997). A mixture of topdressing sand and organic compost consistently reduced dollar spot epidemics over a span of three years. In the same study, when compared to applications of Ringer Compost Plus (CP), a mixture of cow and poultry manure compost and a sludge compost, iprodione provided inferior suppression to dollar spot (Nelson and Craft, 1992). In a later study, Ringer Greens Restore and Ringer CP resulted in greater dollar spot control for up to one month when compared to propiconazole alone.

This, however, changed a month after a second application when propiconazole resulted in better control. Ringer CP was found to have minimal disease suppression (Hoyland and Landschoot, 1993). In a study comparing organic and synthetic fertilizers, organic fertilizers did not enhance disease suppression as urea was found to provide better or equal disease suppression (Landschoot and McNitt, 1997).

It remains unclear whether dollar spot reduction has been achieved due to improved plant growth vigor caused by higher nitrogen levels, alteration of soil pH that destabilizes the pathogen, or microbial interactions with the pathogen (Liu et al., 1995; Landschoot and McNitt, 1997). Increased plant vigor increases the capacity of the turfgrass to outcompete the pathogen, rather than increased plant resistance to the pathogen (Couch and Bloom, 1960). Dependent upon site location, source of nitrogen does not have a consistent effect on the reduction of dollar spot (Townsend et al., 2020). From their study, only nitrogen applications applied every 2 weeks at rates as high as 29.3 kg N ha^{-1} provided consistent reduction in disease severity (Townsend et al., 2020). Ferrous sulphate has also been found to manage dollar spot occurrence in turfgrass systems. Without altering turfgrass quality, summer applications of the fertilizer on a 14-d interval at a rate of 48.8 kg ha^{-1} reduced the severity of dollar spot by >50% (McCall et al., 2016). In contrast, a decrease in overall creeping bentgrass quality when treated with ferrous sulphate was reported by Ervin et al., (2017) When applied with ferrous sulphate, chlorothalonil increased the longevity of dollar spot control than when used alone (Hutchens et al., 2021).

Cultural practices

A variety of cultural practices have been shown to effectively reduce dollar spot symptoms. Cultural management strategies such as decreasing leaf wetness duration, limiting shade, and increasing air circulation, among others, may effectively reduce disease severity on golf courses (Burpee, 1993).

Dew removal by poling and/or morning mowing has been shown to reduce dollar spot (Williams, 1996). Dew and guttation fluid have been considered nutritional sources for disease causing foliar microbes (Williams and Powell, 1995). Rolling putting greens during the morning or afternoon have also been shown to significantly reduce dollar spot incidence and improve turfgrass quality (Nikolai et al., 2001; Giordano et al., 2012).

Rolling, although initially used in the early 20th century to smooth surface imperfections (Beard, 1973), was once abandoned due to concerns of surface compaction. The practice, however, was readopted for competition preparation in the late 1990s to increase ball roll distance. The practice, when done in the morning, has been shown to suppress dollar spot in closely mowed turf due to the action of dew removal from the turfgrass surface (Nikolai et al., 2001; Pigati et al., 2010). Later studies revealed that disease severity was not different within morning-rolled plots than those rolled in the afternoon, despite dew dissipation. In addition, rolling was also found to increase the volumetric water content at the root zone compared to the nontreated plots (Giordano et al., 2012) This suggested that in addition to other unidentified effects of rolling, increase in volumetric water content above 0.25 cm^3 contribute to dollar spot reduction than dew removal alone (McDonald et al., 2006; Giordano et al., 2012). Topdressing with sand was also found to be an effective cultural control option and may contribute up to 50% disease suppression (Green et al., 2019).

Although the reduction of dew has been shown to reduce dollar spot severity, the presence of dew at the time of fungicide application has been met with varying results. Chlorothalonil has been shown to be more effective in controlling dollar spot when applied to a dry canopy than to dew-covered surfaces (McDonald et al., 2006). They found that fungicides were more effective when applied in lower spray volumes (i.e., 409 liters per acre) and postulated that this was likely due to dilution or foliar runoff of the fungicide by dew and/or in high spray volumes (McDonald et al., 2006). However, Huang et al. (2015), found that the presence of dew did not affect fungicide efficacy and suggested that dew removal prior to application was unnecessary.

To reduce mowing frequency, while still maintaining high quality and dense turf, turfgrass managers have turned to the use of plant growth regulators (PGRs). Plant growth regulators that inhibit the synthesis of gibberellic acid (GA) are most commonly applied. In an *in vitro* study, the PGRs flurprimidol and paclobutrazol were found to have a fungistatic effect on *Clarireedia* spp. at lower concentrations than trinexapac-ethyl and may cause increased resistance to demethylation inhibitor fungicides (Burpee et al.,1996, Ok et al., 2011). Field studies also indicated that flurprimidol and paclobutrazol treatments significantly reduced the number of dollar spot epidemics compared to trinexapac-ethyl and a nontreated control. Dollar spot control from applications of iprodione, propiconazole, and chlorothalonil was improved when turf was pretreated with flurprimidol or paclobutrazol. There were inconsistencies between years when evaluating the effect of trinexapac-ethyl on the efficacy of the aforementioned fungicides against dollar spot (Burpee et al., 1996). In contrast, Zhang and Schmidt (2000) found that the application of both trinexapac-ethyl and propiconazole caused significant reduction of dollar spot disease. However, dollar spot severity was decreased when trinexapac-ethyl was used in the absence of fungicides (Huang et al., 2015; Putman and Kaminski., 2011).

Although disease suppression has been shown not to be influenced by mowing frequency, paclobutrazol has been shown to extend the duration of fungicidal control (Putman and Kaminski, 2011; Huang et al., 2015). In a greenhouse study, moisture-stressed turfgrasses had a higher rate of susceptibility to the disease (Couch and Bloom, 1960). Soil moisture is negatively correlated with dollar spot severity. Dollar spot severity has been found to be more severe on a creeping bentgrass fairway subjected to deep and infrequent irrigation (McDonald et al., 2006).

Chemical Control

Although cultural practices may reduce dollar spot incidence and severity, they may not be effective in completely eradicating occurrence of the disease. Therefore, turfgrass managers often supplement cultural practices with fungicides to limit disease development and improve turf quality.

In areas of high disease pressure, nozzle type has been shown to influence fungicide efficacy (Kaminski and Fidanza, 2009). Very coarse and medium droplet sizes, depending on nozzle technology, provided better dollar spot suppression when compared to nozzles delivering extra coarse and fine droplet sizes. Additionally, dollar spot suppression was highest when iprodione or propiconazole were tank mixed with chlorothalonil when compared to when treatments were applied alone (Kaminski and Fidanza, 2009). There have been inconclusive results from studies evaluating the effect of spray application volume when applying fungicides to manage dollar spot. Although with minor differences, chlorothalonil was found to be more effective in dollar spot control at the low application volume of 468 liters/ha) compared to the high volume at 1020 liters/ha (McDonald et al., 2006). In addition, curative applications of propiconazole and chlorothalonil for dollar spot control were not influenced by different spray volumes (McDonald et al., 2006). Similarly, minimal to no differences were observed in a two-year study evaluating the difference in three application volumes (204, 408, or 814 liters/ha) when chlorothalonil was applied as a management strategy to control dollar spot (Kennelly and Wolf, 2009).

Several fungicide classes are registered for use in the management of dollar spot. Bordeaux mixture, made of copper sulphate and lime, was one of the initial fungicides used to manage turfgrass diseases, but was discontinued due to phytotoxic effects on turfgrasses (Cole et al, 1968; Latin 2021). Mercury-based fungicides were then introduced as they had a reduced risk to cause phytotoxicity when they were used appropriately. These were, however, later discontinued due to their toxic nature to human beings (Latin, 2021).

Currently, several fungicide classes are available for use in dollar spot suppression in the turfgrass industry. Arguably one of the most important fungicides in turfgrass disease control is chlorothalonil, a multisite, broad spectrum contact fungicide (Latin, 2021). A single fungicide (thiophanate-methyl) within the benzimidazoles, introduced in the late 1960s, is still registered for use in turfgrass systems (Latin, 2021). Introduced in the 1970s, dicarboximides are currently used to control dollar spot in turf (Detweiler et al., 1983). Iprodione remains the only available active ingredient within this chemical class registered in the United States for turf. Demethylation inhibitors are one of two groups within the sterol biosynthesis mode of action group (Latin, 2021). They are one of the most commonly used chemical classes in turfgrass systems due to their broad-spectrum nature and prominent availability with nine commercially available active ingredients registered for use in turf. Succinate dehydrogenase inhibitors (SDHIs) are fungicides within mode of action group C which target mitochondrial respiration. Out of twenty SDHI fungicides registered under FRAC group seven, five are labelled for dollar spot control (Latin, 2021).

Fungicide Resistance

Over the course of many years, fungicide resistance has developed in at least four chemical classes and has limited the efficacy of available fungicides for the control of dollar spot.

The presence of pathogen-resistant strains of *Clarireedia* spp. in a turfgrass stand is evident by the decline of a fungicide's efficacy over time (Miller, 2002). *Clarireedia* spp. resistance to most fungicides is believed to be quantitatively inherited (Bonos, 2003). Resistance of *Clarireedia* spp. to fungicides within four chemical classes have been observed including quantitative resistance to fungicides within the following classes: succinate dehydrogenase inhibitors (SDHIs), demethylation inhibitors (DMIs), and dicarboximides (Warren et al., 1974; Detweiler et al., 1983; Vargas et al., 1992; Putman et al., 2010; and Stephens and Kaminski, 2019). Qualitative resistance of *Clarireedia* spp. to the benzimidazoles has been confirmed as well.

Resistance is observed when a fungicide that once provided acceptable levels of control of a pathogen becomes less effective or ineffective. When resistance is observed, use of products from different chemical classes or tank-mixing multiple chemical classes is encouraged. However, if using the same products after quantitative resistance is observed, decreased application intervals or increased fungicide rates may still afford acceptable disease suppression. Cross resistance is defined as the development of resistance to different fungicides with a similar mode of action, due to a change in a single genetic factor (Dekker, 1977). In some cases, multi-drug resistance has been found. Multi-drug resistance occurs when an isolate is resistant to fungicides belonging to different chemical classes. The first report of this occurrence in turfgrass was in an isolate of *Clarireedia spp.* that was found to be resistant to iprodione and thiophanate-methyl (Detweiler et al., 1983). Multiple resistance has also been documented across benzimidazoles, DMIs, and dicarboximides (Bishop et al., 2008; Ok et al., 2010; Putman et al., 2010; Stephens and Kaminski 2019). Development and persistence of any resistance could lead to the loss of active ingredients for use in the management of particular pathogens and is therefore crucial for it to be determined and managed at its onset.

Benzimidazoles

Benzimidazole resistance was first observed in isolates collected from New Jersey, Ohio, Pennsylvania, and Illinois in 1972 (Warren et al., 1974). Since the first discovery, resistance to fungicides within this chemical class has been identified in various regions of the United States (Burpee, 1996; Jo et al., 2008; Putman et al., 2010; Stephens and Kaminski, 2019). Resistance to benzimidazoles has been observed to be qualitative, owing to a mutation that changes the pathogen’s protein structure, making it completely insensitive to fungicides in this class (Latin, 2021). Fungicide-resistant isolates were mostly found in fairways compared to roughs and is likely indicative of the relative number of fungicide applications in the respective areas. It was also determined that thiophanate-methyl resistance could not be predicted by the history of application of the fungicide (Koch et al., 2009; Putman et al., 2010; Stephens and Kaminski, 2019).

Dicarboximides

From this broad-spectrum fungicide family, iprodione is the only remaining fungicide for use in the turfgrass industry (Latin, 2021). Iprodione was introduced in the late 1970s in the wake of a ban on the metal-based compounds used as fungicides and increasing resistance of the *Clarireedia* spp. to benzimidazoles (Warren et al., 1974). Iprodione is considered to have a broad-spectrum mode of action due to its ability to interfere with signal transduction that interrupts the pathogen’s infection process of germ tube elongation and appressorium formation (Latin, 2021; Yamaguchi and Fujimura, 2005). Insensitivity to iprodione was first identified in an isolate collected from a golf course in Michigan (Detweiler et al., 1983). Resistance to fungicides in this group has been associated with a mutation in the coiled-coil region of the histidine kinas gene (*BOS-1* gene) (Ma and Michailides, 2005).

Demethylation inhibitor (DMI) fungicides

Demethylation inhibitors were introduced in 1979 and remain widely used in the USA (Golembiewski et al., 1995). Fungicides within this chemical class are the most widely used turfgrass fungicides (Latin, 2021). DMIs move acropetally and inhibit biosynthesis of ergosterol in fungi, by inhibiting the demethylation of oxidative sterol (Siegel, 1981). They were used for over a decade before a record of insensitivity within turfgrass pathogens was observed. Reduced sensitivity to DMIs can be partially determined by analysis of the application frequency of the fungicide (Koch et al., 2009). Cross resistance is common among DMIs (Hsiang et al., 1997). Pathogen populations that have exhibited reduced sensitivity to one DMI will be cross resistant to other active ingredients within the chemical class (Koller and Scheinpflug, 1987).

Field resistance to DMIs has been investigated for the last three decades with the first report in the early 1990s (Golembiewski et al., 1995). In 1997, Hsiang et al., carried out a study on DMI sensitivity on pathogen populations that had been collected in the Summer of 1994 before the fungicide class was legally introduced in Canada for turfgrass use. Despite wide variation in sensitivity amongst the sampled populations, seven out of eight populations were very sensitive to the DMIs. A single population, collected near the USA border, was suspected to have been exposed to DMIs before their legal introduction. A follow up study with nine populations of previously studied *Clarireedia* spp., revealed that five had been exposed to DMIs in the subsequent years and were found to have reduced sensitivity to propiconazole (Hsiang et al., 2006). Isolates whose sensitivity had decreased after repeated use of DMIs, were able to overcome fungicide efficacy within fourteen days in the field (Hsiang et al., 2006).

Succinate Dehydrogenase Inhibitors (SDHI) Fungicides

The SDHI fungicides work by interfering with the delivery of succinate derived electrons into the ubiquinone pool (Janssen et al., 1997). Interference with this process inhibits the formation of adenosine triphosphate (ATP). In recent years, fungicides in this group have been widely accepted for use in the turfgrass industry as an alternative to the continued use of existing DMIs and QoIs. Following their widespread use in recent years, resistance was first documented in 2018 (Popko et al., 2018). A single nucleotide polymorphism mutation was determined to be the cause of resistance in *Clarireedia* spp. The succinate dehydrogenase complex is made of four sub units (SdhA, SdhB, SdhC and SdhD). Despite increased sensitivity to fluopyram, isolates with a B-H267Y mutation were found to exhibit cross resistance to boscalid and penthipyrad. Two isolates with a silent mutation on the SdhB gene exhibited cross resistance to isofetamid, penthiopyrad and boscalid (Popko et al., 2018).

Fungicides and Turfgrass Phytotoxicity

Sterol biosynthesis inhibiting compounds (SBIs) is a mode of action group within which DMIs are classified. When these compounds are used as fungicides for the management of various diseases in cropping systems, they have been found to have non-target effects, both positive and negative. Field applications of propiconazole on broadleaf weeds led to reduced growth. This was confirmed with *in vitro* studies where redroot pigweed (*Amaranthus retroflexus*) seedling lengths were significantly reduced (Hanson et al., 2003). Negative non-target effects include small dark-colored leaves, germination reduction, and shortened root internodes and stems (Biggs, 1990; Kane and Smiley, 1983). A chemical treatment is said to be phytotoxic when it has retardation effects on the plant’s growth pattern. Phytotoxicity has been reported from different chemical treatments, such as mercury and copper-based products and the Bordeaux mixture (Latin, 2021). These products cause a scorching effect on foliage. Some products have been found to induce color changes to a bluish-green hue while others may cause yellowing of the turfgrass when applied. Over the years, DMI fungicides have been found to have predominant plant growth regulation effects. (Buchenauer and Röhner, 1981; Kane and Smiley, 1983; Fletcher et al., 1986;)

Most DMI fungicides have both fungistatic and plant growth regulatory effects when applied on plants. The commonly observed trait after DMI application in turfgrasses is darkening of the foliage (Kane and Smiley, 1983). These fungicides also interfere with the synthesis of gibberellic acid (GA) thereby reducing cell elongation of the host (Bigelow et al., 1995). Propiconazole and triadimefon are triazole compounds that work as biostimulants and hence regulate physiological processes in plants (Fletcher et al., 1986). Propiconazole elicits development of wider leaf blades and stunts growth while causing a bluish hue (Dernoeden, 2002). Several studies have found propiconazole to cause an increase in clipping yield weight when used in the field while other DMIs caused a reduction in the weight (Kane and Smiley, 1983; McCullough et al., 2006). Temperature and precipitation were found to have an influence on the growth regulatory effects of DMIs (Pennypacker et al., 1982). Field experiments with triazoles were found to give varying results when compared to experiments done with the same fungicides within growth chambers (Kane and Smiley, 1983). Growth chamber evaluations have consistently shown that DMIs reduce root mass, but these findings are inconsistent in field studies (Kane and Smiley, 1983; Bigelow et al., 1995).

There have been market claims that recently introduced DMIs have little to no phytotoxic effects on turfgrasses, ‘prothioconazole provides dollar spot control without causing turf thinning or injury (Bayer, 2022) : Mefentrifluconazole can be sprayed on any turf and any season (BASF 2022). Although many studies have investigated the scope of resistance to fungicides among

Clarireedia spp., few studies have looked closely at variation in resistance and phytotoxicity among commercially available active ingredients within the DMI class. Therefore, the objectives of this research are to: 1) elucidate differences in turfgrass phytotoxicity among DMI fungicides on two cool-season grass species; 2) determine discriminatory doses and EC_{50} values for nine demethylation-inhibiting fungicides for *C. jacksonii* isolates with varying levels of insensitivity; and 3) identify potential differences in fungicide efficacy on dollar spot disease among DMIs in the field.

CHAPTER II. PHYTOTOXICITY OF COMMERCIALLY AVAILABLE DEMETHYLATION-INHIBITING FUNGICIDES WHEN APPLIED TO AN ANNUAL BLUEGRASS (*POA ANNUA*) AND CREEPING BENTGRASS (*AGROSTIS STOLONIFERA*) FAIRWAY

Introduction

Demethylation inhibitor fungicides (DMIs) are classified under the mode of action of sterol biosynthesis inhibiting (SBI) compounds (Latin, 2021). There have been reports of both positive and negative non-target impacts of SBIs in agricultural systems. Positive effects include delayed leaf senescence, minimal chlorophyll degradation, increased visual quality, and increased drought and cold tolerance. Conversely, negative effects include shortened stem and root internodes, leaf epinasty, and abscission (Biggs, 1990; Kane and Smiley, 1983). These fungicides are broad spectrum chemistries commonly used to suppress various diseases in turfgrass systems (Latin, 2021).

Demethylation inhibitors act by inhibiting the ergosterol biosynthesis in the cell wall of fungi and disrupting the activity of gibberellin hormones within the plant (Bigelow et al., 1995). Gibberellins are key plant hormones that regulate elongation, germination, and flower development in plants (Schwechheimer, 2012). Fungicides within the DMI chemical class have been found to inhibit sterol biosynthesis as well as gibberellin activity. Triadimefon caused significant reduction in the growth of primary leaves and roots of barley (*Hordeum vulgare* L.) grown under low light conditions (Buchenauer and Röhner, 1981). However, the addition of gibberellins reverted the retardation. Extracted shoot tissues of the same barley plants were found to have reduced gibberellic-like activity when compared to the nontreated seedlings (Buchenauer and Röhner, 1981).

Demethylation inhibitor fungicides are commonly used in the treatment of dollar spot in a variety of turfgrass systems. Although DMIs have been effective in disease control, non-target effects on both annual bluegrass (*Poa annua* L.) and creeping bentgrass (*Agrostis stolonifera* L.) have been reported (Buchenauer and Grossman, 1977; Reicher and Throssell, 1997). Repeated applications of DMI fungicides have resulted in phytotoxic effects on turfgrasses when applied during the summer months. Phytotoxic symptoms observed include dark green foliage and wider leaf blades (Buchenauer and Grossman, 1977; Kane and Smiley, 1983). Propiconazole was observed to cause a darkening of foliage and decreased visual quality of the turfgrass surface on a creeping bentgrass putting green (Reicher and Throssell, 1997). Studies evaluating the effect of propiconazole have shown an increase in clipping yield following repeated applications. However, this has not been true for other DMI fungicides (Pennypacker et al., 1982; Kane and Smiley, 1983). Triadimefon applications resulted in reduced clipping weights in annual bluegrass only when the turfgrass was subjected to either extreme heat or wet conditions compared to other environmental conditions (Pennypacker et al., 1982). In a greenhouse study, the triazole compounds fenarimol, nuarimol, and triadimefon were found to reduce root weights and leaf growth rates in Kentucky bluegrass (*Poa pratensis* L.). The fungicides also caused an increase in chlorophyll retention (Kane and Smiley, 1983).

Triazole compounds, such as propiconazole and triadimefon, regulate physiological processes due to their capacity to behave as biostimulants. Triadimefon has a triazole group and chlorophenyl similar to the PGR paclobutrazol (Fletcher et al., 1986). Bermudagrass treated with high-rate applications of fenarimol resulted in reduced root mass but had no impact to root length (McCullough et al., 2007). In a study to evaluate the effects of DMI fungicides on creeping bentgrass rooting depth and clipping yield, none of the DMI fungicide applications caused significant effects (Mitkowski and Chaves, 2013). In another field study, propiconazole applied alone or alternated with chlorothalonil was found to increase clipping weight (Reicher and Throssell, 1997).

Although phytotoxic effects caused by DMIs have been characterized in several studies, most have focused on a limited number of active ingredients (i.e., propiconazole and triadimefon). Market claims by manufacturers purport that recently introduced DMIs have less potential to cause turfgrass injury. Therefore, the objectives of this study were to 1) evaluate variation in turfgrass injury among nine commercially available DMIs when applied to annual bluegrass and creeping bentgrass fairways and 2) elucidate the ability of two application volumes to mitigate turfgrass injury.

Materials and Methods

In 2020, two field trials were initiated at the Valentine Turfgrass Research Centre located in University Park, PA. Trials were conducted on creeping bentgrass (CBG) and annual bluegrass (ABG) research fairways. Trials were repeated in 2021. Soil at both trial sites was a Hagerstown silt loam with a pH of 6.3 and 4.96% organic matter and a pH of 6.6 and 5.25% organic matter for the ABG and CBG sites, respectively. Both sites were mowed 3 times per week and irrigated as necessary. Plots measured 0.9 m x 1.8 m and were arranged as a two-way factorial in a randomized complete block design with four replications.

For treatments, one factor included ten fungicides applied on a 14-day interval. Fungicides applied in the trial included propiconazole (1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole) (Banner MAXX, Syngenta Crop Protection, Greensboro, NC), mefentrifluconazole (2*RS*)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1*H*-1,2,4-

triazol-1-yl) propan-2-ol), (Maxtima, BASF corporation, Research Triangle Park, NC), myclobutanil (a-butyl-a-(chlorophenyl)-1H-1,2,4,triazole-1-propanenitrile) (Eagle, Dow AgroSciences LLC, Indianapolis, IN), flutriafol (α-(2-fluorophenyl)-α-(4-fluorophenyl)-1H-1,2,4-triazole-1-ethanol) (Rayora, FMC Corporation, Philadelphia PA), triadimefon (1-(4-Chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanone (Bayleton, Bayer Environmental Science, Research Triangle Park, NC), tebuconazole (α–[2-(4-chlorophenyl)ethyl]-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol) (Torque, NuFarm Americas Inc, Alsip, IL), metconazole (5-[(4-chlorophenyl)methyl]-2,2-dimethyl-1-(1 H-1,2,4-triazol-1-ylmethyl) (Tourney, NuFarm Americas Inc, Alsip, IL), triticonazole (1,2-benzisothiazol-3(2H)-one) (Trinity, BASF, Research Triangle Park, NC), prothioconazole, (2-[2-{1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-3-thione (Densicor, Bayer Environmental Science, Research Triangle Park, NC), a non-DMI fungicide control pydiflumetofen, 1H-Pyrazole-4-carboxamide,3-(difluoromethyl)-N-methoxy-1-methyl-N-[1-methyl-2-(2,4,6 trichlorophenyl) ethyl] (Posterity, Syngenta Crop Protection, Greensboro, NC) and a nontreated control plot. Prothioconazole was only applied in 2021 when it became commercially available in the turf market. Fungicide treatments were applied on the following dates: 25 Jun, 9 Jul, 23 Jul, 6 Aug, and 19 Aug 2020 and 15 Jun, 29 Jun, 13 Jul, 27 Jul, and 10 Aug 2021. All treatments and application rates, application volumes, and application dates are listed in Table 2.1. A second factor included application volume in which all treatments were applied at either 409 or 818 L ha^{-1} of water in a factorial treatment design. Treatments were applied using a CO_2 pressurized handheld sprayer fitted with AI11004EVS and AI10008EVS air induction flat fan nozzles (TeeJet Technologies, Wheaton, IL) calibrated to deliver 409 or 818 L ha^{-1} at 276 kPa, respectively. Temperature and precipitation data was collected from US Climate data website .

Plots were visually rated for quality on a scale of 1 to 9, where 1 = entire plot brown or dead, 6 = minimum level of acceptable turfgrass quality for a golf course fairway, and 9 = optimum greenness and density. To determine any phytotoxicity caused by the fungicide treatments, foliar turfgrass injury, based on deviations in color, texture, density and growth) was visually rated on a scale of 0 to 5, where 0 = no turfgrass injury, 2 = maximum level of acceptable foliar turfgrass injury for a golf course fairway, and 5 = entire plot brown or dead. All data were subjected to analysis of variance using the PROC MIXED model in SAS (SAS Institute Inc., Cary, NC, USA) and means separate using Fisher's Protected least significant difference test at $P \leq 0.05$.

Results

Treatment applications (Table 2.1), as well as ratings, were not conducted on the same dates in 2020 and 2021, therefore, all data were analyzed separately by year. Across all years and turfgrass species, few interactions between application volume and fungicides were observed and application volume influenced foliar injury on only a few select dates. The influence of fungicide, however, was significant on most dates.

Creeping Bentgrass. In 2020, creeping bentgrass foliar injury was first observed following the second application of fungicides. On 16 Jul, 21-days after the initial treatment (DAIT), foliar injury ranged from 0.0 to 1.8 and no treatments exhibited unacceptable injury (Table 2.2). Two weeks after the second application, however, plots treated with propiconazole, triadimefon, and myclobutanil had injury ranging from 2.3 to 2.8 and were considered unacceptable (> 2.0). Across all rating dates where injury was present, turfgrass treated with triadimefon had the greatest injury on 6 dates. Within plots treated with triadimefon and myclobutanil, turfgrass injury remained at or slightly above unacceptable levels (> 2.0) through early-August. Despite a fifth application of all products on 19 Aug, injury decreased within all plots for the remainder of the study. On all rating dates, mefentrifluconazole had the lowest injury (< 0.1) when compared to all treatments and the non-treated control. Turfgrass within plots treated with the DMIs flutriafol, metconazole, triticonazole and the non-DMI fungicidal control pydiflumetofen exhibited the lowest injury and was always less than the drought-induced injury observed within the nontreated control plots on all rating dates. Except for the first rating date (16 Jul), tebuconazole also had equivalent or lower injury than the nontreated control.

No fungicide x application volume interactions were present on any rating date. The main effect of application volume only influenced injury ratings on 20 Aug, with treatments applied in 818 L ha^{-1} resulting in slightly higher injury (0.7) when compared to treatments applied at 409 L ha^{-1} (0.4) (Table 2.2).

In 2021, creeping bentgrass injury was not observed until approximately 4 weeks after the initial application (Table 2.3). On the first two dates in which injury was observed, a significant fungicide x application volume interaction was present. On 12 Jul, plots treated with propiconazole, and applied at 409 L ha^{-1}, resulted in the highest injury (1.0) (Fig 2.1). No differences, however, were observed among treatments applied at the higher application volume. By 19 Jul, propiconazole and triadimefon applied at the low application volume had the highest injury ratings (2.0 to 2.5), whereas no other fungicide resulted in unacceptable turfgrass injury. When applied at higher application volumes (i.e., 818 L ha^{-1}), propiconazole continued to express the greatest injury (2.5). Moderate injury (1.3) was observed within plots treated with myclobutanil, but no significant injury was observed among all other fungicides when applied at the higher application volume. The main effect of injury was significant from 26 Jul until the last date on which injury was observed (i.e., 16 Aug). Although propiconazole, triadimefon, and myclobutanil resulted in the highest injury (1.1 to 1.6) on various rating dates, none of the fungicides resulted in unacceptable injury ratings after 19 July. Little to no injury was observed in all other fungicide treatments and the nontreated control.

Turfgrass quality data was influenced by both injury from the treatments and the presence of dollar spot. In 2020, differences in turfgrass quality were first observed 21 DAIT (Table 2.4). In general, quality was reduced within plots exhibiting the greatest turfgrass injury. Turf treated with triadimefon had the lowest quality when compared across all rating dates and treatments but was only considered unacceptable on a single rating date (22 Jul). Few differences in turfgrass quality were observed among treatments within 1 week after the final application. Application volume influenced quality on a single rating date (20 Aug) where higher application volumes resulted in reduced turfgrass quality (Table 2.4). In 2021, turfgrass quality was at or just below acceptable within all treated plots 3 weeks after initiating the trial and no differences were observed among treatments (Table 2.5). Quality within the nontreated control was generally unacceptable throughout the trial. In general, all fungicide-treated plots exhibited acceptable quality throughout the study. However, turfgrass quality within triticonazole-treated plots was unacceptable (5.3) on 19 Jul, approximately 1 week following the third application. In addition to the influence of treatments, reduction in plot quality was also influenced by the outbreak of dollar spot disease (Table 2.6).

Annual bluegrass. In 2020, the main effect of fungicide was significant on all rating dates and application volume influenced injury on two rating dates (Table 2.7). By 8 Jul, turfgrass within plots treated with triticonazole exhibited unacceptable injury (2.7). Injury within triticonazole-treated plots ranged from 2.0 and 3.7 until 20 Aug. Over the duration of the study, plots treated with metconazole, tebuconazole, and myclobutanil exhibited unacceptable or maximum acceptable (2.0) injury on 4, 3 and 1 rating dates, respectively. Minor, but acceptable, injury was observed in all other fungicide-treated plots and the nontreated control. Turfgrass foliar injury within all treatments had waned and was considered acceptable 1 wk after the final application. By 3 Sept, all treatments had injury ratings less than 1.0. Although only significant on two days, fungicides applied in higher volumes (818 L ha^{-1}) resulted in higher injury to annual bluegrass as compared to fungicides applied in lower volumes (Table 2.7).

In 2021, injury to annual bluegrass was generally less than that observed in 2020. Injury was first observed on 29 Jun, but gradually increased within select plots until peaking in mid-Jul (Table 2.8). Plots treated with metconazole and triticonazole generally had the highest injury, but injury was only considered unacceptable on two rating dates within plots treated with metconazole. Injury started to decline within all plots following the third application and was considered acceptable despite continued applications.

A single fungicide x application volume interaction was observed on the final rating date (9 Aug). Although only minor injury was observed at this time, plots treated with triticonazole in the lowest application volume and myclobutanil at the highest application volume resulted in the greatest injury (Table 2.9).

Turfgrass quality in annual bluegrass varied among treatments in 2020. Plots treated with triticonazole generally were considered unacceptable (< 6.0) on most rating dates when treatments were being applied (Table 2.10). Turfgrass quality within mefentrifluconazole plots was consistently higher or similar to the nontreated control plots on all rating dates (Table 2.10). With few exceptions, all other treatments resulted in acceptable quality. Towards the end of the study, leaf spot, caused by *Drechlera poae* (Baudys) Shoemaker, began to develop within the study site resulting in reduced quality. While most fungicides suppressed the disease, applications of pydiflumetofen did not control the leaf spot outbreak which resulted in unacceptable quality (Table

2.11). Turfgrass quality within plots treated with pydiflumetofen decreased rapidly after leaf spot disease set in during the second week of August. Propiconazole and triadimefon applications resulted in wider leaf blades and a dark green surface despite the plots appearing injured

In 2021, on annual bluegrass, reduction in turfgrass quality was first observed 21 d after initial applications, but all treatments were considered acceptable (> 6.0) (Table 2.12). Unacceptable turfgrass quality was observed 28 and 35 DAIT within plots treated with metconazole. There was variation in quality levels among other treatments, but all remained above the minimum acceptable quality. Two turfgrass diseases occurred during the trial and were evaluated as shown in Fig 2.2.

Discussion

In both years, and across the two turfgrass species, foliar injury to the two turfgrass species following repeated applications of the DMI fungicides varied. Regardless of turfgrass species or year, injury generally first appeared three to four weeks after the initial application.

When applied to creeping bentgrass, propiconazole, triadimefon, and myclobutanil resulted in the greatest injury and regulation. On both turfgrass species, injury to the turfgrass appeared to be more severe in 2020 when compared to 2021. Turfgrass injury can be caused by environmental factors, mechanical activity, and chemical application to turfgrass surfaces. Exposure of *Poa pratensis* (Kentucky bluegrass) and *Schedonorus arundinaceus* (Schreb.) Dumort (tall fescue) to high temperatures (30 to 35^{o}C) and drought, have been shown to cause injury and decrease turfgrass quality (Jiang and Huang, 2001).

Total rainfall recorded from the first to the last day of treatment application was 50 mm and 193 mm for 2020 and 2021, respectively (Fig 2.3). Higher rainfall and lower average daily temperatures during the trial period in 2021 may have influenced turfgrass injury and quality when compared to 2020 (Fig 2.1). Temperature ranged from 14 to 28 ^{o}C for both years (Fig 2.3). In 2020 of this study, foliar injury to the non-treated control plots appeared to be the result of drought stress. However, heightened injury was observed in several DMI-treated plots. Enhanced injury in 2021, in the absence of drought stress, indicates foliar injury observed in both years resulted directly from the DMI fungicides.

In this study, triticonazole was found to cause significant foliar injury to annual bluegrass, while injury was not observed in creeping bentgrass. Propiconazole caused injury to both annual bluegrass and creeping bentgrass. Propiconazole and triadimefon also resulted in a darkening or a bluish hue of the foliage and a widening of the leaf blades. The turfgrass stand, however, still appeared to be injured or regulated. This observation coincides with previous studies that showed presence of wider leaf blades and chlorophyll increase causing dark green foliage (Buchenauer and Grossman, 1977; Kane and Smiley, 1983). Triadimefon has also been found to reduce transpiration rates in wheat, leaving the plants shorter and with thicker greener leaves than nontreated plants (Fletcher and Nath, 1984). Reports of reduced transpiration, leads to the hypothesis that plants treated with the fungicides would behave in a similar manner despite the precipitation levels. When evaluating the effectiveness of triadimefon and fenarimol against dollar spot after rain events, Couch (1985) found that irrigation prior to drying after fungicide application reduces the effectiveness of the fungicides by less than 50%. However, the fungicides' effectiveness was not affected by irrigation applied soon after the chemical was dried on the turfgrass foliage (Couch, 1985). Although studies indicate that rain events can have an impact on fungicide activity, no rain events occurred in our study for ≥ 3 hrs following fungicide applications.

In 2020, mefentrifluconazole seemed to improve turfgrass quality and plots exhibited less foliar injury than the nontreated control plots on both CBG and ABG fairways. Annual bluegrass quality, however, did decline in pydiflumetofen-treated plots due to leaf spot. While most DMI fungicides result in minor to moderate turfgrass injury, it is unclear if mefentrifluconazole reduced foliar injury from drought when compared to drought stress observed within the nontreated control. Future research may be warranted to evaluate the influence of various DMI fungicides on foliar injury and turfgrass quality when applied during periods of drought stress.

Turfgrass managers are encouraged to consider various factors when selecting fungicides for use on different turfgrass species. An alternation of active ingredients from different FRAC groups may help to avoid turfgrass injury or disease outbreaks.

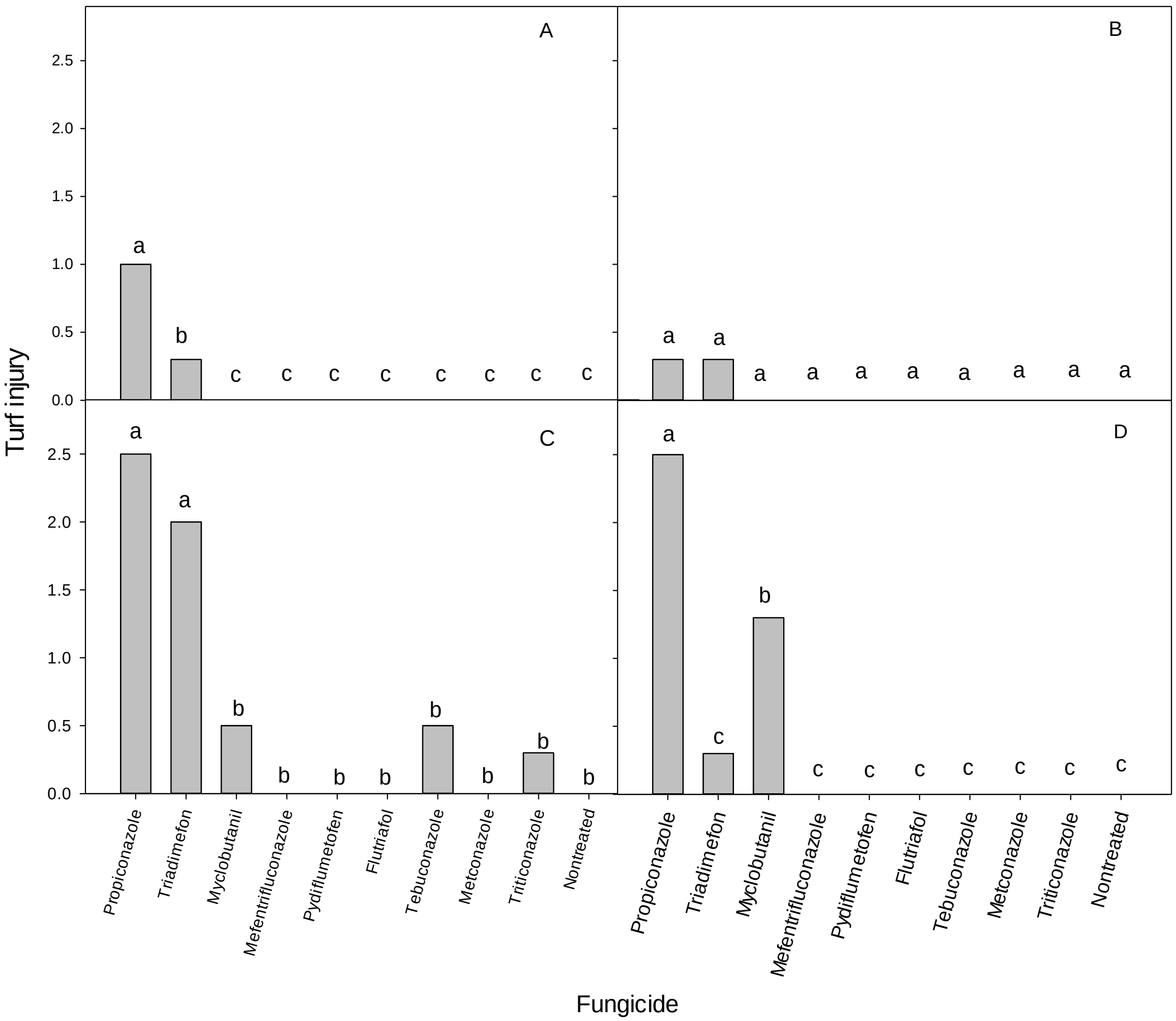

Figure 2.1. Fungicide x application volume interactions were observed for creeping bentgrass foliar injury on two dates in 2021. Foliar injury to creeping bentgrass was observed on 12 Jul when applied at 409 L/ha (A) or 818 L/ha (B) and on 19 Jul when applied at 409 L/ha (C) or 818 L/ha (D). Foliar turfgrass injury was visually assessed on a 0 to 5 scale based on deviations in color, texture, density, and growth), where 0 = no turfgrass injury, 2 = maximum level of acceptable turfgrass injury for a golf course fairway, and 5 = entire plot brown or dead. Within each injury rating, means followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

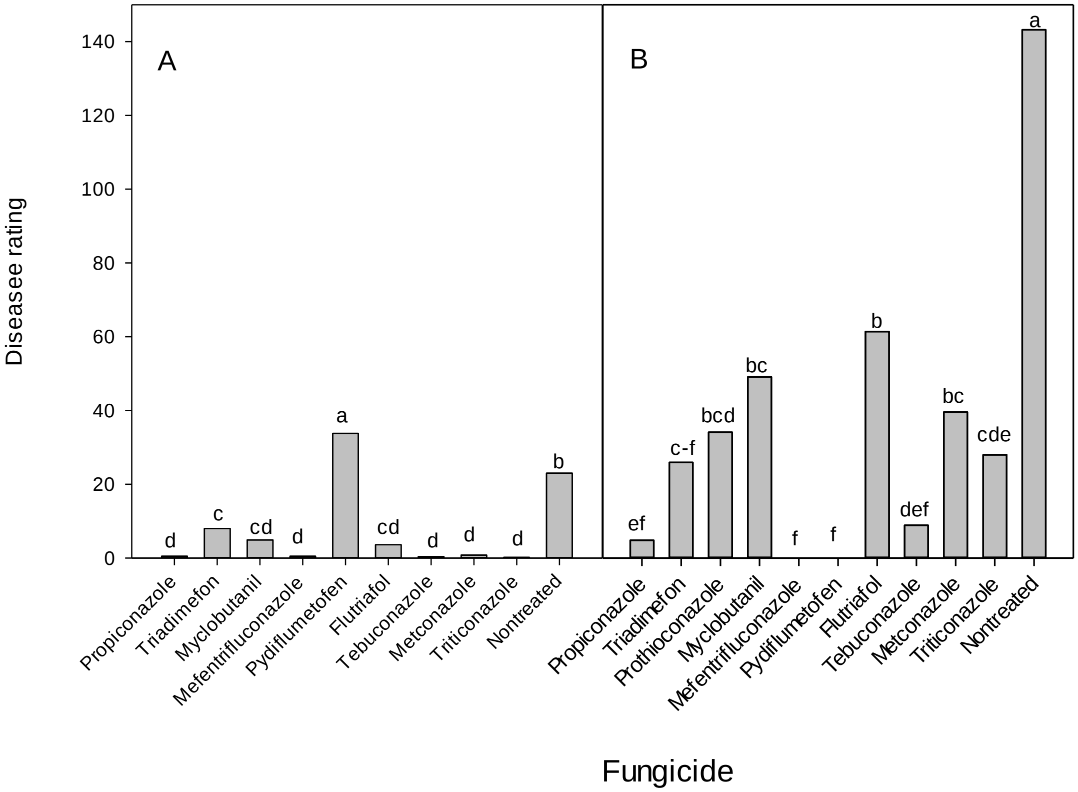

Figure 2.2. Leaf spot severity on an annual bluegrass fairway in August 2020 (A) and dollar spot severity on a creeping bentgrass fairway in August 2021 (B). Leaf spot was visually assessed on a scale 0 to 100 percent where 0 = no disease symptoms present and 100 = entire plot covered by leaf spot. Dollar spot severity was assessed by counting the number of dollar spot infection centers within each plot. Within each disease rating, means followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

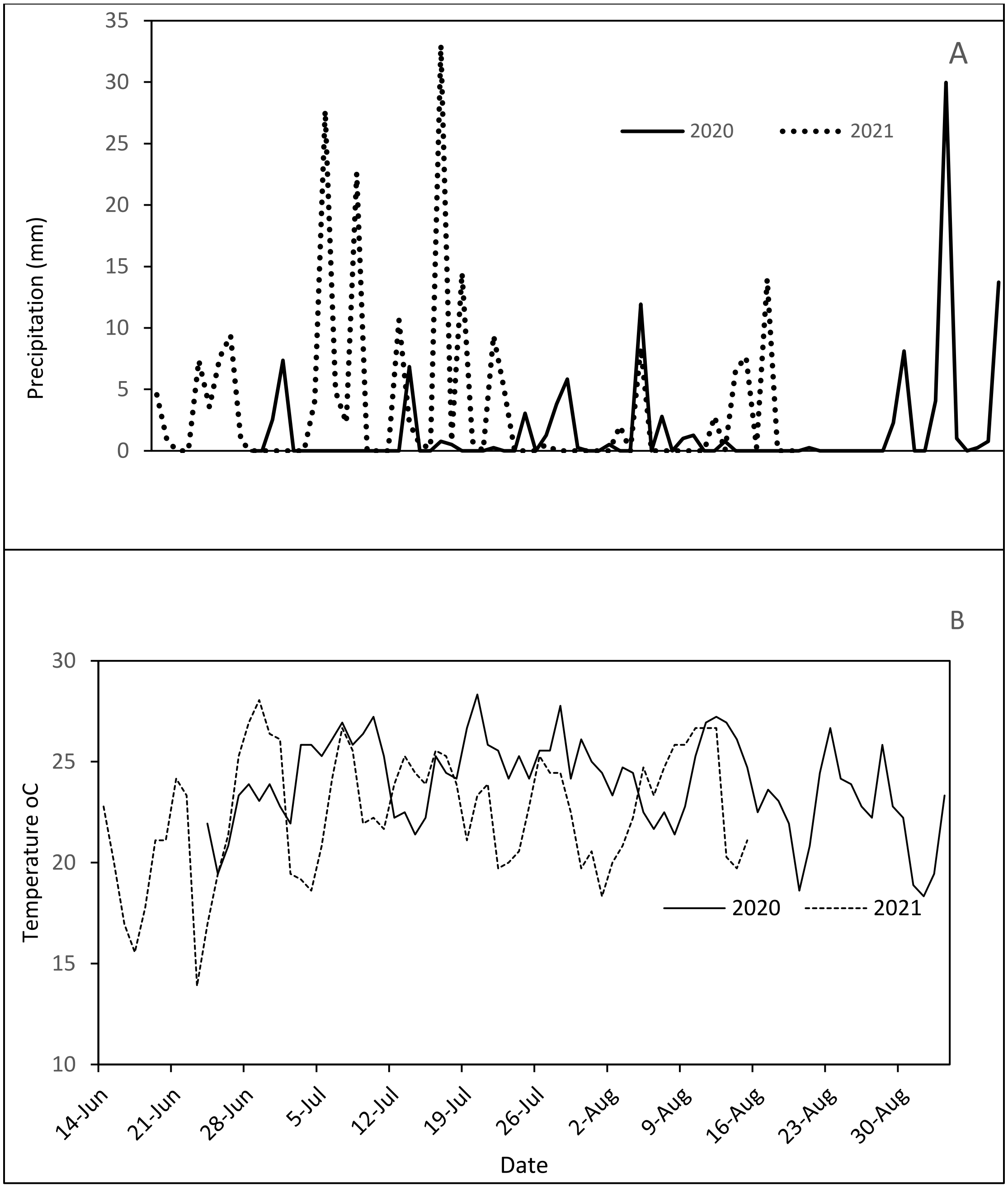

Figure 2.3. Total daily precipitation (A) and average daily air temperature (B) throughout the trial periods in 2020 and 2021.

Table 2.1. Active ingredients and rates of demethylation inhibiting and succinate dehydrogenase inhibiting fungicides applied to creeping bentgrass and annual bluegrass research fairways to assess turfgrass injury and quality.

Active ingredient[z]	Application rate (L ha^{-1})
Propiconazole	6.4
Triadimefon	3.2
Myclobutanil	7.6
Prothioconazole[y]	0.6
Mefentrifluconazole	1.3
Pydiflumetofen	0.5
Flutriafol	4.5
Tebuconazole	3.5
Metconazole	1.2
Triticonazole	6.4

[z] Fungicide treatments were applied on the following dates: 25 Jun, 9 Jul, 23 Jul, 6 Aug, and 19 Aug 2020 and 15 Jun, 29 Jun, 13 Jul, 27 Jul, and 10 Aug 2021. Treatments were applied in 409 L ha^{-1} and 818 L ha^{-1} of water in a factorial treatment design.

[y] Prothioconazole was applied 2021 trials only when it became commercially available for turfgrass use in the United States.

Table 2.2. Creeping bentgrass foliar injury following repeated applications of various demethylation inhibiting fungicides and pydiflumetofen to a research fairway at the Joseph Valentine Turfgrass Research Center, 2020.

	Turfgrass injury[z]								
Treatment[y]	16 Jul	22 Jul	30 Jul	6 Aug	1 Aug	20 Aug	27 Aug	3 Sep	10 Sep
Fungicide									
Propiconazole	1.0 bc[x]	2.3 a	1.1 bc	1.8 ab	1.3 bc	1.0 ab	0.6 ab	1.0 a	0.1 a
Triadimefon	1.8 a	2.8 a	2.0 a	2.3 a	2.1 a	0.9 ab	0.1 c	0.0 b	0.0 a
Myclobutanil	1.5 ab	2.3 a	1.3 b	2.0 ab	2.0 a	1.4 a	0.8 a	0.0 b	0.1 a
Mefentrifluconazole	0.0 e	0.0 d	0.0 e	0.0 f	0.1 d	0.0 d	0.0 c	0.0 b	0.0 a
Pydiflumetofen	0.3 de	0.8 c	0.3 de	0.3 ef	0.1 d	0.0 d	0.0 c	0.0 b	0.0 a
Flutriafol	0.4 de	0.9 bc	0.4 de	0.4 ef	0.3 d	0.1 cd	0.0 c	0.0 b	0.0 a
Tebuconazole	1.4 ab	1.4 bc	0.6 cd	1.0 cd	0.8 cd	0.5 bcd	0.0 c	0.0 b	0.0 a
Metconazole	0.3 de	1.0 bc	0.5 de	0.8 de	0.4 d	0.1 cd	0.0 c	0.0 b	0.0 a
Triticonazole	0.5 c-e	0.8 c	0.5 de	0.6 de	0.5 d	0.4 bcd	0.0 c	0.0 b	0.0 a
Nontreated	0.8 cd	1.5 b	1.1 bc	1.5 bc	1.5 ab	0.8 abc	0.3 c	0.0 b	0.0 a
Volume									
409 L ha^{-1}	-	-	-	-	-	0.4 b	-	-	-
818 L ha^{-1}	-	-	-	-	-	0.7 a	-	-	-
Source of variation									
Fungicide (F)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0005	0.0004	<0.0001	0.4998
Volume (V)	1.0000	0.3546	0.0887	0.7035	0.0527	0.0330	0.0959	0.3584	0.1517
F*V	0.7082	0.7899	0.6988	0.9350	0.2197	0.6560	0.5148	0.5679	0.4998

[z] Turfgrass foliar injury was visually assessed on a 0 to 5 scale, where 0 = no turfgrass injury, 2 = maximum level of acceptable turfgrass injury for a golf course fairway, and 5 = entire plot brown or dead.

[y] Treatments were applied on 25 Jun, 9 Jul, 23 Jul, 6 Aug and 19 Aug 2020.

[x] Means in a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

Table 2.3. Creeping bentgrass foliar injury following repeated applications of various demethylation inhibiting fungicides and pydiflumetofen to a research fairway at the Joseph Valentine Turfgrass Research Center, 2021.

	Turfgrass injury[z]					
Treatment[y]	12 Jul	19 Jul	26 Jul	2 Aug	9 Aug	16 Aug
Fungicide						
Propiconazole	0.6 a[x]	2.5 a	1.6 a	1.5 a	1.1 a	1.0 a
Triadimefon	0.3 a	1.1 a	0.8 b	1.4 a	0.9 ab	0.3 b
Prothioconazole	0.0 a	0.0 a	0.6 bc	0.0 b	0.3 c	0.0 b
Myclobutanil	0.0 a	0.9 a	0.3 bcd	0.1 b	1.3 a	0.1 b
Mefentrifluconazole	0.0 a	0.0 a	0.1 cd	0.0 b	0.0 c	0.0 b
Pydiflumetofen	0.0 a	0.0 a	0.1 cd	0.0 b	0.0 c	0.0 b
Flutriafol	0.0 a	0.0 a	0.1 cd	0.0 b	0.4 bc	0.0 b
Tebuconazole	0.0 a	0.3 a	0.0 d	0.0 b	0.0 c	0.1 b
Metconazole	0.0 a	0.0 a	0.0 d	0.0 b	0.1 c	0.0 b
Triticonazole	0.0 a	0.1 a	0.0 d	0.0 b	0.3 c	0.1 b
Nontreated	0.0 a	0.0 a	0.0 d	0.0 b	0.0 c	0.0 b
Volume						
409 L ha^{-1}	-	-	-	-	-	-
818 L ha^{-1}	-	-	-	-	-	-
Source of variation						
Fungicide (F)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Volume (V)	0.0865	0.1133	0.5339	0.1952	1.0000	0.7824
F*V	0.0034	0.0014	0.6531	0.1686	0.8417	0.9899

[z] Turfgrass foliar injury was visually assessed on a 0 to 5 scale, where 0 = no turfgrass injury, 2 = maximum level of acceptable turfgrass injury for a golf course fairway, and 5 = entire plot brown or dead.

[y] Treatments were applied on a 14-day interval for a total of five treatments on 15 Jun, 29 Jun, 13 Jul, 27 Jul, and 10 Aug 2021.

[x] Means in a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

Table 2.4. Turfgrass quality on a creeping bentgrass fairway following repeated applications of various demethylation inhibiting fungicides and pydiflumetofen to a research fairway at the Joseph Valentine Turfgrass Research Center, 2020.

	Turfgrass quality[z]				
Treatment[y]	16 Jul	22 Jul	30 Jul	6 Aug	13 Aug
Fungicide					
Propiconazole	7.1 bc[x]	6.9 abc	7.3 abc	6.6 bcd	7.6 ab
Triadimefon	6.3 d	5.6 d	6.1 d	6.0 e	6.1 d
Myclobutanil	6.3 d	6.5 bc	6.8 c	6.3 de	6.4 cd
Mefentrifluconazole	7.8 a	7.0 ab	7.8 a	7.6 a	7.9 a
Pydiflumetofen	7.4 ab	6.6 abc	7.6 a	6.8 bcd	7.5 ab
Flutriafol	6.8 cd	6.9 abc	7.4 ab	7.1 ab	7.0 bc
Tebuconazole	7.5 ab	6.9 abc	7.3 abc	7.1 ab	7.4 ab
Metconazole	7.3 abc	7.1 a	7.4 ab	6.9 bc	7.4 ab
Triticonazole	7.3 abc	6.4 c	7.0 bc	6.4 cde	6.6 cd
Nontreated	7.3 abc	7.0 ab	7.4 ab	6.9 bc	7.9 a
Volume					
409 L ha^{-1}	-	-	-	-	-
818 L ha^{-1}	-	-	-	-	-
Source of variation					
Fungicide (F)	<0.0001	0.0003	0.0001	<0.0001	<0.0001
Volume (V)	0.7164	0.8550	0.8550	0.0516	0.3497
F*V	0.6177	0.9830	0.7641	0.4692	0.5407

[z] Turfgrass quality was visually assessed on a 0 to 9 scale, where 1 = entire plot brown or dead, 6 = minimum level of acceptable turfgrass quality and 9 = optimum greenness and density for a golf course fairway.

[y] Treatments were applied on a 14-day interval for a total of five treatments on 25 Jun, 9 Jul, 23 Jul, 6 Aug, and 19 Aug 2020.

[x] Means in a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

Table 2.4 (cont.). Turfgrass quality on a creeping bentgrass fairway following repeated applications of various demethylation inhibiting fungicides and pydiflumetofen to a research fairway, 2020.

	Turfgrass quality[z]				
Treatment[y]	20 Aug	27 Aug	3 Sep	10 Sep	17 Sep
Fungicide					
Propiconazole	7.6 ab	8.0 a	6.9 d	6.4 f	7.1 b
Triadimefon	6.8 c	8.0 ab	7.5 bc	7.5 bcd	7.0 bc
Myclobutanil	6.8 c	8.1 a	7.4 c	6.8 ef	7.0 bc
Mefentrifluconazole	8.0 a	7.8 ab	8.0 a	8.1 a	8.5 a
Pydiflumetofen	7.9 a	7.5 ab	7.9 ab	8.0 ab	7.4 b
Flutriafol	7.4 abc	7.9 ab	7.9 ab	7.4 cd	7.5 b
Tebuconazole	7.8 a	7.8 a	8.0 a	7.6 abc	6.8 bc
Metconazole	7.8 a	7.6 ab	7.8 abc	7.6 abc	7.5 b
Triticonazole	7.0 bc	7.4 b	7.8 abc	7.4 cd	6.3 c
Nontreated	7.6 ab	7.8 ab	7.5 bc	7.0 de	8.9 a
Volume					
409 L ha^{-1}	7.7 a	-	-	-	-
818 L ha^{-1}	7.3 b	-	-	-	-
Source of variation					
Fungicide (F)	0.0011	0.0429	<0.0001	<0.0001	<0.0001
Volume (V)	0.0110	0.1911	0.1340	0.7096	0.2598
F*V	0.7308	0.8860	0.7924	0.5825	0.6569

[z] Turfgrass quality was visually assessed on a 0 to 9 scale, where 1 = entire plot brown or dead, 6 = minimum level of acceptable turfgrass quality and 9 = optimum greenness and density for a golf course fairway.

[y] Treatments were applied on a 14-day interval for a total of five treatments on 25 Jun, 9 Jul, 23 Jul, 6 Aug, and 19 Aug 2020.

[x] Means in a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

Table 2.5. Turfgrass quality on a creeping bentgrass fairway following repeated applications of various demethylation inhibiting fungicides and pydiflumetofen to a research fairway at the Joseph Valentine Turfgrass Research Center, 2021.

	Turfgrass quality[z]						
Treatment[y]	6 Jul	12 Jul	19 July	26 Jul	2 Aug	9 Aug	16 Aug
Fungicide							
Propiconazole	5.6 a	7.1 bc	7.3 bc	8.1 a	7.6 abc	7.0 bc	7.8 a
Triadimefon	5.9 a	7.1 bc	6.8 c	7.3 bcd	6.8 d	7.0 bc	7.3 a
Prothioconazole	5.6 a	7.5 abc	6.8 c	7.5 a-d	7.1 cd	8.0 a	7.1 a
Myclobutanil	6.3 a	6.9 c	8.1 a	6.9 d	7.1 cd	6.6 c	7.3 a
Mefentrifluconazole	6.4 a	8.1 a	8.0 a	7.8 abc	8.3 a	8.0 a	7.9 a
Pydiflumetofen	6.4 a	7.9 ab	6.6 c	7.9 ab	8.0 a	8.0 a	7.9 a
Flutriafol	5.9 a	7.4 abc	8.0 a	6.9 d	6.8 d	7.4 abc	7.4 a
Tebuconazole	5.9 a	7.8 ab	7.6 ab	7.8 abc	7.9 ab	7.9 a	7.9 a
Metconazole	6.1 a	7.3 bc	7.1 bc	7.1 cd	6.9 d	7.8 ab	7.4 a
Triticonazole	6.1 a	7.3 bc	7.0 bc	7.5 a-d	7.3 bcd	7.4 abc	7.1 a
Nontreated	5.6 a	6.0 d	5.3 d	5.3 e	4.5 e	4.3 d	5.0 b
Volume							
409 L ha^{-1}	-	-	-	-	-	-	-
818 L ha^{-1}	-	-	-	-	-	-	-
Source of variation							
Fungicide (F)	0.4752	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Volume (V)	0.5350	0.4048	0.3249	0.8767	0.4653	1.0000	0.6950
F*V	0.4953	0.9740	0.6809	0.9912	0.6066	0.5769	0.8304

[z] Turfgrass quality was visually assessed on a 0 to 9 scale, where 1 = entire plot brown or dead, 6 = minimum level of acceptable turfgrass quality and 9 = optimum greenness and density for a golf course fairway.

[y] Treatments were applied on a 14-day interval for a total of five treatments on 15 Jun, 29 Jun, 13 Jul, 27 Jul, and 10 Aug, 2021.

[x] Means in a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

Table 2.6. Dollar spot severity on a creeping bentgrass fairway following applications of various demethylation inhibiting fungicides and pydiflumetofen to a research fairway at the Joseph Valentine Turfgrass Research Center, 2021.

	Dollar spot infection centers (DSIC)[z]						
Treatment[y]	6 Jul	12 Jul	19 July	26 Jul	2 Aug	9 Aug	16 Aug
Fungicide							
Propiconazole	-	23 def	10 def	7 de	4 cd	1 d	7 ef
Triadimefon	-	42 bcd	21 bcd	32 bc	5 cd	2 d	38 c-f
Prothioconazole	-	31 cde	16 de	24 cd	23 bc	7 cd	50 bcd
Myclobutanil	-	49 bc	34 bc	48 b	37 b	24 b	72 bc
Mefentrifluconazole	-	8 fg	2 ef	3 de	0 d	0 d	0 f
Pydiflumetofen	-	2 g	0 f	0 e	0 d	0 d	0 f
Flutriafol	-	58 b	36 b	42 bc	40 b	21 bc	90 b
Tebuconazole	-	12 efg	4 ef	4 de	1 d	0 d	13 def
Metconazole	-	35 cd	25 bcd	37 bc	39 b	12 bcd	58 bc
Triticonazole	-	35 cd	20 cd	33 bc	12 cd	5 cd	41 cde
Nontreated	-	110 a	114 a	142 a	199 a	192 a	210 a
Volume							
409 L ha^{-1}	-	-	-	-	-	-	-
818 L ha^{-1}	-	-	-	-	-	-	-
Source of variation							
Fungicide (F)	0.2851	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Volume (V)	0.9264	0.6739	0.5684	0.9927	0.5706	0.1666	0.8383
F*V	0.7721	0.9763	0.9814	0.8792	0.8699	0.9799	0.8096

[z] Dollar spot severity was assessed by counting the number of dollar spot infection centers within each plot.

[y] Treatments were applied on a 14-day interval for a total of five treatments on 15 Jun, 29 Jun, 13 Jul, 27 Jul, and 10 Aug 2021.

[x] Means in a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher’s protected least significant difference test.

Table 2.7. Annual bluegrass foliar injury following repeated applications of various demethylation inhibiting fungicides and pydiflumetofen to a research fairway at the Joseph Valentine Turfgrass Research Center, 2020.

	Turfgrass injury[z]				
Treatment[y]	2 Jul	8 Jul	16 Jul	22 Jul	30 Jul
Fungicide					
Propiconazole	0.8 ab[x]	1.8 ab	1.8 b	1.5 bcd	1.7 cd
Triadimefon	1.0 a	1.6 b	1.7 b	1.6 bc	1.5 cd
Myclobutanil	0.8 abc	1.6 ab	1.9 ab	1.8 bc	2.2 bc
Mefentrifluconazole	0.3 bcd	1.2 b	0.8 c	0.9 cd	0.7 e
Pydiflumetofen	0.0 d	1.2 b	1.0 c	0.6 d	1.1 de
Flutriafol	0.5 a-d	1.5 b	0.9 c	1.2 bcd	1.1 de
Tebuconazole	0.4 bcd	1.8 ab	1.6 b	2.0 b	1.9 bc
Metconazole	0.2 cd	1.4 b	2.0 ab	2.2 b	2.3 b
Triticonazole	0.2 cd	2.7 a	2.6 a	3.7 a	3.5 a
Nontreated	0.1 d	1.2 b	0.7 c	0.8 cd	1.1 de
Volume					
409 L ha^{-1}	-	-	-	1.4 b	1.5 b
818 L ha^{-1}	-	-	-	1.9 a	1.9 a
Source of variation					
Fungicide (F)	0.0076	0.0404	<0.0001	<0.0001	<0.0001
Volume (V)	0.6489	0.1184	0.1454	0.0152	0.0130
F*V	0.6091	0.7000	0.5696	0.1147	0.7601

[z] Turfgrass foliar injury was visually assessed on a 0 to 5 scale where 0 = no turfgrass injury, 2 = maximum level of acceptable turfgrass injury for a golf course fairway and 5 = entire plot brown or dead.

[y] Treatments were applied on a 14-day interval for a total of five treatments on 25 Jun, 9 Jul, 23 Jul, 6 Aug, and 19 Aug 2020.

[x] Means in a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

Table 2.7. (cont.). Annual bluegrass foliar injury following repeated applications of various demethylation inhibiting fungicides and pydiflumetofen to a research fairway at the Joseph Valentine Turfgrass Research Center, 2020.

	Turfgrass injury[z]				
Treatment[y]	6 Aug	13 Aug	20 Aug	27 Aug	3 Sep
Fungicide					
Propiconazole	1.4 bc	1.7 b[x]	0.4 cd	0.4 bcd	0.3 b
Triadimefon	0.4 d	1.7 b	1.1 bc	0.2 bcd	0.1 b
Myclobutanil	1.1 cd	1.8 b	1.6 ab	0.7 ab	0.9 a
Mefentrifluconazole	0.4 d	0.2 c	0.2 d	0.0 d	0.0 b
Pydiflumetofen	0.2 d	0.3 c	0.6 bcd	0.4 a-d	0.0 b
Flutriafol	0.3 d	0.3 c	0.4 cd	0.0 cd	0.0 b
Tebuconazole	1.8 bc	2.1 b	1.4 ab	0.5 abc	0.0 b
Metconazole	2.1 b	1.9 b	0.6 bcd	0.9 a	0.0 b
Triticonazole	3.3 a	3.0 a	2.0 a	0.6 ab	0.2 b
Nontreated	0.2 d	0.2 c	0.3 d	0.1 bcd	0.0 b
Volume					
409 L ha^{-1}	-	-	-	-	-
818 L ha^{-1}	-	-	-	-	-
Source of variation					
Fungicide (F)	<0.0001	<0.0001	0.0001	0.0123	0.0038
Volume (V)	0.4263	0.3777	0.3196	0.7932	0.9146
F*V	0.2878	0.5595	0.9991	0.1388	0.9440

[z] Turfgrass foliar injury was visually assessed on a 0 to 5 scale, where 0 = no turfgrass injury, 2 = maximum level of acceptable turfgrass injury for a golf course fairway, and 5 = entire plot brown or dead.

[y] Treatments were applied on a 14-day interval for a total of five treatments on 25 Jun, 9 Jul, 23 Jul, 6 Aug, and 19 Aug 2020.

[x] Means in a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

Table 2.8. Annual bluegrass foliar injury following repeated applications of various demethylation inhibiting fungicides and pydiflumetofen to a research fairway at the Joseph Valentine Turfgrass Research Center, 2021.

	Turfgrass injury[z]						
Treatment[y]	29 Jun	6 Jul	12 Jul	19 Jul	26 Jul	2 Aug	9 Aug
Fungicide							
Propiconazole	0.3 ab[x]	0.6 c	1.0 c	0.9 bcd	0.3 b	0.5 bc	0.6 a
Triadimefon	0.0 b	0.5 c	0.5 d	0.5 cde	0.0 b	0.1 c	0.0 a
Prothioconazole	0.0 b	0.0 d	0.0 e	0.3 de	0.0 b	0.1 c	0.0 a
Myclobutanil	0.0 b	0.5 c	0.4 de	0.6 cde	0.1 b	0.5 bc	0.8 a
Mefentrifluconazole	0.0 b	0.0 d	0.0 e	0.1 e	0.0 b	0.1 c	0.0 a
Pydiflumetofen	0.0 b	0.0 d	0.0 e	0.4 de	0.0 b	0.1 c	0.0 a
Flutriafol	0.0 b	0.0 d	0.0 e	0.4 de	0.0 b	0.0 c	0.0 a
Tebuconazole	0.0 b	1.4 b	1.1 c	1.1 bc	0.0 b	0.9 ab	0.1 a
Metconazole	0.4 a	1.9 a	2.4 a	2.5 a	0.9 a	1.5 a	0.3 a
Triticonazole	0.4 a	1.5 ab	1.9 b	1.5 b	0.9 a	1.4 a	0.9 a
Nontreated	0.0 b	0.0d	0.0 e	0.5 cde	0.8 a	0.0 c	0.0 a
Volume							
409 L ha^{-1}	-	-	-	-	-	-	-
818 L ha^{-1}	-	-	-	-	-	-	-
Source of variation							
Fungicide (F)	0.0040	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Volume (V)	0.4173	0.1714	0.3613	0.0562	0.2615	0.3134	0.7239
F*V	0.4266	0.3971	0.9507	0.3799	0.8884	0.9944	0.0119

[z] Turfgrass foliar injury was visually assessed on a 0 to 5 scale, where 0 = no turfgrass injury, 2 = maximum level of acceptable turfgrass injury for a golf course fairway, and 5 = entire plot brown or dead.

[y] Treatments were applied on a 14-day interval for a total of five treatments on 15 Jun, 29 Jun, 13 Jul, 27 Jul, and 10 Aug 2021.

[x] Means in a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

Table 2.9. Fungicide by application volume interactions on annual bluegrass foliar injury following repeated applications of various demethylation inhibiting fungicides and pydiflumetofen to a research fairway at the Joseph Valentine Turfgrass Research Center, 2021.

	Turfgrass injury[z]	
	9 August	
Treatment[y]	409 L ha^{-1}	818 L ha^{-1}
Fungicide		
Propiconazole	0.8 b[x]	0.5 b
Triadimefon	0.0 d	0.3 b
Myclobutanil	0.5 bc	1.0 a
Mefentrifluconazole	0.0 d	0.0 c
Pydiflumetofen	0.0 d	0.0 c
Flutriafol	0.0 d	0.0 c
Tebuconazole	0.3 cd	0.0 c
Metconazole	0.0 d	0.5 b
Triticonazole	1.3 a	0.5 b
Nontreated	0.0 d	0.0 e

[z] Turfgrass foliar injury was visually assessed on a 0 to 5 scale, where 0 = no turfgrass injury, 2 = maximum level of acceptable turfgrass injury for a golf course fairway, and 5 = entire plot brown or dead.

[y] Treatments were applied on a 14-day interval for a total of five treatments on 15 Jun, 29 Jun, 13 Jul, 27 Jul, and 10 Aug 2021.

[x] Means in a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

Table 2.10. Turfgrass quality on an annual bluegrass fairway following repeated applications of various demethylation inhibiting fungicides and pydiflumetofen to a research fairway at the Joseph Valentine Turfgrass Research Center, 2020.

	Turfgrass quality[z]						
Treatment[y]	2 Jul	8 Jul	16 Jul	22 Jul	30 Jul	6 Aug	13 Aug
Fungicide							
Propiconazole	6.5 b[x]	6.2 bc	6.3 cd	6.6 ab	6.5 bcd	6.2 bc	6.7 bc
Triadimefon	6.5 b	6.5 ab	6.8 abc	6.2 abc	7.0 ab	6.5 ab	6.6 bc
Myclobutanil	6.4 b	5.8 bc	6.8 abc	6.2 abc	6.4 bcd	5.8 bc	6.2 cde
Mefentrifluconazole	7.1 a	6.3 ab	7.3 a	7.0 a	7.3 a	6.4 ab	7.9 a
Pydiflumetofen	7.3 a	6.8 a	6.9 ab	6.8 ab	6.9 abc	6.8 a	5.9 def
Flutriafol	6.8 ab	6.4 ab	6.9 ab	6.5 ab	7.0 ab	6.4 ab	7.0 b
Tebuconazole	6.6 b	6.1 bc	6.6 bc	6.0 bc	6.4 cd	6.1 bc	6.1 cde
Metconazole	7.0 ab	6.4 ab	6.6 bc	6.0 bc	5.9 d	6.4 ab	6.3 cd
Triticonazole	6.8 ab	5.6 c	5.8 d	5.5 c	5.3 e	5.6 c	5.4 f
Nontreated	7.3 a	6.3 ab	7.2 a	6.5 ab	6.8 abc	6.3 ab	5.6 ef
Volume							
409 L ha^{-1}		6.4 a	-	-	-	-	-
818 L ha^{-1}	-	6.1 b	-	-	-	-	-
Source of variation							
Fungicide (F)	0.0072	0.0189	<0.0001	0.0003	<0.0001	<0.0001	<0.0001
Volume (V)	0.3281	0.0430	0.5012	0.2072	0.1406	0.1826	0.5482
F*V	0.6730	0.2914	0.6581	0.7868	0.8152	0.6944	0.8865

[z] Turfgrass quality was visually assessed on a 0 to 9 scale, where 1 = entire plot brown or dead, 6 = minimum level of acceptable turfgrass quality and 9 = optimum greenness and density for a golf course fairway.

[y] Treatments were applied on a 14-day interval for a total of five treatments on 25 Jun, 9 Jul, 23 Jul, 6 Aug, and 19 Aug 2020.

[x] Means in a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

Table 2.10 (cont.). Turfgrass quality on an annual bluegrass fairway following repeated applications of various demethylation inhibiting fungicides and pydiflumetofen to a research fairway at the Joseph Valentine Turfgrass Research Center, 2020.

	Turfgrass quality[z]				
Treatment[y]	20 Aug	27 Aug	3 Sep	10 Sep	17 Sep
Fungicide					
Propiconazole	7.4 ab	7.3 ab	8.0 a	7.6 bc	7.9 bc
Triadimefon	6.2 de	6.4 cd	7.2 b	7.5 c	7.3 d
Myclobutanil	6.3 cde	5.9 de	6.9 bc	7.3 cd	7.2 d
Mefentrifluconazole	7.9 a	7.8 a	8.0 a	8.3 a	8.8 a
Pydiflumetofen	5.5 f	5.6 e	6.2 d	6.4 e	7.1 d
Flutriafol	6.7 bcd	7.1 abc	7.8 a	7.6 bc	7.9 c
Tebuconazole	6.9 bc	7.1 bc	7.9 a	8.0 ab	8.5 a
Metconazole	7.5 a	6.7 bcd	8.0 a	8.0 ab	8.4 ab
Triticonazole	6.1 ef	7.2 ab	7.7 a	8.0 ab	8.6 a
Nontreated	5.8 ef	5.7 e	6.5 cd	7.0 d	6.8 d
Volume					
409 L ha^{-1}	-	-	-	-	-
818 L ha^{-1}	-	-	-	-	-
Source of variation					
Fungicide (F)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Volume (V)	0.4965	0.6348	0.4232	0.6432	0.4248
F*V	0.6626	0.6159	0.4875	0.4992	0.2739

[z] Turfgrass quality was visually assessed on a 0 to 9 scale, where 1 = entire plot brown or dead, 6 = minimum level of acceptable turfgrass quality and 9 = optimum greenness and density for a golf course fairway.

[y] Treatments were applied on a 14-day interval for a total of five treatments on 25 Jun, 9 Jul, 23 Jul, 6 Aug, and 19 Aug 2020.

[x] Means in a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

Table 2.11. Leaf spot severity on an annual bluegrass putting green following repeated applications of demethylation inhibitor fungicide and pydiflumetofen to a research fairway at the Joseph Valentine Turfgrass Research Center, 2020.

	Percent leaf spot[z]							
Treatment[y]	6 Aug	13 Aug	20 Aug	27 Aug	3 Sep	10 Sep	17 Sep	23 Sep
Fungicide								
Propiconazole	0.4 bc[x]	0.1 b	0.5 d	4.6 c	3.6 cde	0.3 c	0.0 b	0.0 b
Triadimefon	4.9 b	3.8 b	8.0 c	5.4 c	6.0 bc	2.2 b	1.6 b	0.1 b
Myclobutanil	3.0 bc	4.2 b	4.9 cd	5.4 c	5.9 bcd	1.4 bc	0.9 b	0.5 b
Mefentrifluconazole	0.3 bc	0.6 b	0.5 d	0.5 c	0.0 e	0.0 c	0.4 b	0.0 b
Pydiflumetofen	20.0 a	25.6 a	33.8 a	24.0 a	15.2 a	7.3 a	3.5 a	1.4 a
Flutriafol	3.2 bc	2.7 b	3.6 cd	2.7 c	2.2 de	0.0 c	0.4 b	0.2 b
Tebuconazole	0.1 bc	0.0 b	0.4 d	0.0 c	0.0 e	0.0 c	0.0 c	0.0 b
Metconazole	0.1 bc	0.3 b	0.8 d	0.8 c	0.9 e	0.0 c	0.2 b	0.0 b
Triticonazole	0.2 c	0.1 b	0.2 d	2.5 c	0.0 e	0.2 c	0.0 b	0.3 b
Nontreated	15.4 a	21.4 a	23.0 b	15.7 b	9.5 b	6.7 a	4.8 a	1.8 a
Volume								
409 L ha^{-1}	-	-	-	-	-	-	-	-
818 L ha^{-1}	-	-	-	-	-	-	-	-
Source of variation								
Fungicide (F)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Volume (V)	0.9607	0.5672	0.3997	0.4690	0.3078	0.2945	0.9939	0.7419
F*V	0.3235	0.6873	0.6179	0.4442	0.0852	0.7096	0.9831	0.2241

[z] Leaf spot severity was visually assessed on a scale 0 to 100 percent where 0 = no disease symptoms present and 100 = entire plot covered by leaf spot.

[y] Treatments were applied on a 14-day interval for a total of five treatments on 25 Jun, 9 Jul, 23 Jul, 6 Aug, and 19 Aug 2020.

[x] Means in a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

Table 2.12. Turfgrass quality on an annual bluegrass fairway following repeated applications of various demethylation inhibiting fungicides and pydiflumetofen to a research fairway at the Joseph Valentine Turfgrass Research Center, 2021.

	Turfgrass quality[z]						
Treatment[y]	6 Jul	12 Jul	19 July	26 Jul	2 Aug	9 Aug	16 Aug
Fungicide							
Propiconazole	7.4 c	7.9 a	7.0 bc	7.9 ab	7.5 ab	7.6 ab	7.4 bcd
Triadimefon	7.4 c	8.4 a	7.5 ab	7.9 ab	7.8 a	8.0 a	7.9 ab
Prothioconazole	7.9 ab	7.9 a	7.6 ab	7.9 ab	7.9 a	7.9 a	8.0 a
Myclobutanil	7.5 bc	7.9 a	7.9 a	8.0 a	7.5 ab	7.1 c	7.0 cd
Mefentrifluconazole	8.0 a	8.3 a	7.6 ab	8.0 a	7.8 a	8.0 a	7.9 ab
Pydiflumetofen	8.0 a	8.0 a	7.6 ab	8.0 a	7.8 a	7.9 a	8.0 a
Flutriafol	7.9 ab	8.1 a	7.8 a	8.0 a	7.8 a	7.9 a	8.0 a
Tebuconazole	6.4 d	7.1 b	7.0 bc	7.5 b	7.1 b	7.4 bc	7.5 abc
Metconazole	6.1 d	5.9 c	5.8 d	6.8 c	7.1 b	7.0 cd	7.0 cd
Triticonazole	6.5 d	6.8 b	6.5 c	7.0 c	6.5 c	6.6 d	6.9 d
Nontreated	8.0 a	8.3 a	7.5 ab	8.0 a	7.5 ab	8.0 a	7.9 ab
Volume							
409 L ha^{-1}	-	-	-	-	-	-	-
818 L ha^{-1}	-	-	-	-	-	-	-
Source of variation							
Fungicide (F)	<0.0001	<0.0001	<0.0001	<0.0001	0.0011	<0.0001	<0.0001
Volume (V)	1.0000	0.6139	0.1148	0.7925	0.3921	0.4457	0.1413
F*V	0.1056	0.6081	0.3803	0.5950	0.2342	0.0176	0.6068

[z] Turfgrass quality was visually assessed on a 0 to 9 scale, where 1 = entire plot brown or dead, 6 = minimum level of acceptable turfgrass quality and 9 = optimum greenness and density for a golf course fairway.

[y] Treatments were applied on a 14-day interval for a total of five treatments on 15 Jun, 29 Jun, 13 Jul, 27 Jul, and 10 Aug 2021.

[x] Means in a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

CHAPTER III. DETERMINATION OF DISCRIMINATORY DOSES FOR NINE DEMETHYLATION-INHIBITING FUNGICIDES FOR *CLARIREEDIA JACKSONII* ISOLATES AND VARIATION IN MYCELIAL GROWTH ACROSS ACTIVE INGREDIENTS.

Introduction

The fungal pathogen causing dollar spot in turfgrasses was recently reclassified into the family Rutstroemiaceae and separated into five unique species within the genus *Clarireedia* (F.T. Bennett) L.A. Beirn, B.B. Clarke, C. Salgado, and J.A. Crouch (Salgado-Salazar et al., 2018; Hu et al., 2019). Four initially described species include *C. jacksonii*, *C. monteithiana*, *C. homoeocarpa*, and *C. Benetti* (Salgado-Salazar et al., 2018). Following the initial reclassification, a fifth species, *C. paspali*, was identified on seashore paspalum (*Paspalum vaginatum*) in China (Hu et al., 2019).

Management of dollar spot epidemics involves various cultural and chemical management strategies. In situations requiring fungicides, repeated applications are often needed to adequately suppress the disease. These applications may result in the reduction of sensitivity in pathogen populations requiring turfgrass managers to change fungicide classes used, increase the quantity of fungicides applied, or reduce the application intervals to adequately control the disease. Fungicide resistance occurs because of genetic mutations as a result of the pathogen's effort to overcome the fungicide's inhibition of its functions (Latin, 2021). *Clarireedia* species have developed resistance to fungicides within five chemical classes including the benzimidazoles, dicarboximides, heavy metals, demethylation inhibitors (DMIs), and succinate dehydrogenase inhibitors (SDHIs).

Resistance to the heavy metals was first documented in the 1960s (Cole et al., 1968). Ultimately, heavy metals were removed from use in turfgrass due to their toxicity to humans and negative impact on the environment. This was followed by the release of benzimidazoles, the first site-specific fungicide class for use in the turfgrass market. Qualitative resistance was quickly observed as the fungus mutated to create a tubulin protein that the fungicide was unable to bind to (Goldberg and Cole, 1973; Latin, 2021). Cross resistance within the benzimidazole family was also reported, where *Clarireedia* (formerly *Sclerotinia homoeocarpa*) isolates resistant to thiophanate-methyl exhibited a similar response to benomyl (Warren et al., 1974).

The next chemical class introduced for dollar spot control in the turfgrass industry was the dicarboximides. Within the turfgrass industry, iprodione is the only remaining registered dicarboximide. This broad-spectrum fungicide family was introduced in the late 1970s and field resistance first documented in 1982 (Detweiler and Vargas, 1983). Although laboratory trials indicated isolate insensitivity to iprodione, the fungicide remains effective against the pathogen in field trials (Jo et al., 2006) and continues to be used by turfgrass managers today.

Demethylation inhibitors (DMIs) were first introduced to turfgrass managers in the 1980s. Fungicides within this chemical class interfere with ergosterol biosynthesis, disrupting the development and function of fungal cell membranes. Field resistance within the class was first identified in the early 1990s (Vargas et al., 1992). An *in vitro* assay confirmed reports of insensitivity a few years later (Golembiewski et al., 1995). Fungal populations collected in Canada were found to have a wide variation in fungicide sensitivity when evaluated against propiconazole. One of the eight sampled populations had reduced sensitivity to propiconazole, despite being collected prior to the introduction of DMIs into the turfgrass market in Canada. It was suspected that the population had been exposed to the fungicide class as it had been collected near the USA border (Hsiang et al., 1998).

Succinate dehydrogenase inhibitors (SDHIs) is the latest fungicide class introduced into the turfgrass market, becoming commercially available around 2004 (Latin, 2021). Fungicides within the SDHIs target fungal respiration sites which inhibit the production of adenosine triphosphate by blocking the transport of electrons within complex two of the process (Latin, 2010). Currently, fungicides in this group have been widely accepted for use in the turfgrass industry as an alternative to the repeated use of DMIs and QoIs. Following their widespread use in turf since their initial introduction, resistance to boscalid, penthiopyrad and isofetamid was documented in 2018 (Popko et al., 2018). A single nucleotide polymorphism mutation was determined to be the cause of resistance in *Clarireedia* spp. (Popko et al., 2018).

Of the chemical classes available for control of dollar spot in turf, DMI fungicides are among the most studied as it relates to fungicide resistance. Jo et al. (2006) characterized the resistance of *Clarireedia* spp. in Ohio. Out of 55 sampled populations, eighteen had reduced sensitivity to propiconazole. Miller et al. (2002) indicated a significant relationship with in planta control of dollar spot and *in vitro* suppression of *Clarireedia* spp. using propiconazole. After a field study and *in vitro* analysis in New England, isolates collected from populations previously exposed to propiconazole exhibited higher relative mycelial growth (RMG) values than a baseline population (Popko et al., 2012). Determined EC_{50} values, identified as a 50% inhibition of fungal mycelial growth relative to a non-amended control across a series of fungicide titrations, have been used as an indicator of fungicide insensitivity (Golembiewski et al., 1995; Hsiang et al., 1998; Miller et al., 2002; Popko et al., 2012).

Another method to assess fungicide sensitivity within fungal isolates is through the use of discriminatory doses. To determine discriminatory doses, isolates are grown on media amended with fungicides at different concentrations. Relative mean growth and EC_{50} values are calculated, and log EC_{50} values regressed against the RMGs at each fungicide concentration. The concentration that yields the highest R^2 is considered the discriminatory dose (Jo et al., 2006; Putman et al., 2010; Stephens and Kaminski, 2019).

Whereas determination of the EC_{50} values for an individual isolate of a pathogen requires screening the pathogen across numerous titration rates of a particular fungicide, the use of a single discriminatory dose for a particular fungicide can significantly reduce the time required to screen isolates. Determining the discriminatory doses for individual fungicides and specific pathogens eliminates the need to determine EC_{50} values for every isolate whose fungicide sensitivity level needs to be determined. Previous research has documented the discriminatory dose concentration of propiconazole to be 0.1 ug a.i. ml^{-1} (Jo et al., 2006; Stephens and Kaminski, 2019).

Demethylation inhibiting fungicides comprise the largest number of individual active ingredients available for use in turf, with ten commercially available fungicides as stand-alone products or in pre-mixes. Despite the large number of unique active ingredients, most research studies have utilized propiconazole when investigating *Clarireedia* spp. insensitivity among the FRAC code 3 fungicides (FRAC, 2021).

Since their introduction, differences in the duration of dollar spot control and anecdotal evidence suggesting variation in disease suppression among fungicides within the DMIs has been reported. Despite these reports, there is no known research that has investigated differences in pathogen suppression among the commercially available fungicides within this class. The objectives of this study, therefore, were to: 1) identify EC_{50} and RMG values for *Clarireedia* spp.

across nine commercially available DMI fungicides; 2) determine the discriminatory dose for each of these fungicides; and 3) elucidate differences among isolates with varying degrees of sensitivity to propiconazole across all commercially available DMI fungicides.

Materials and Methods

An *in vitro* experiment was carried out to determine the discriminatory dose of all commercially available DMI fungicides. A total of 50 isolates were used to determine the EC_{50} and RMG values of *Clarireedia jacksonii* to nine DMI fungicides and ultimately to determine the discriminatory dose of each active ingredient.

Selection and growth of isolates. Hyphal tipped cultures of fifty *Clarireedia* spp. isolates were randomly drawn from a collection of over 1000 isolates with varying levels of insensitivity to propiconazole (Stephens and Kaminski, 2019). Potato dextrose agar (PDA) plugs containing mycelium of known isolates were extracted from storage in a 4^{o}C refrigerator (VWR-Avantor, Radnor, PA). Prior to extraction, isolates had been stored at 4°C in 2.0 mL microcentrifuge tubes containing 1 mL sterile de-ionized water.

Isolates were grown on antibiotic water agar (AWA) for 5 d prior to transferring to PDA. The AWA was prepared by adding 10 g of bacteriology agar (Difco Laboratories, Detroit, MI) to 500 ml of distilled water and the mixture sterilized in an autoclave for 20 min at 125^{o}C. After cooling media to 56^{o}C in a water bath (Thermo Fischer Scientific, Waltham, MA), 0.25 g of streptomycin sulfate (Tokyo Chemical Industry, Portland, OR) and 0.25 g Penicillin G potassium (EMB Millipore Corporation, Billerica, MA) were added to prevent bacterial growth. Potato dextrose agar was prepared by adding 19.5 g of potato dextrose agar (HI Media Laboratories, Mumbai, India) to 500 ml of distilled water and autoclaving at 125^{o}C for 20 min. The molten solution was cooled to 56°C in a water bath, poured into 100 mm x 15 mm petri dishes, and allowed to solidify in a sterile biosafety hood for 24 h prior to use.

EC_{50}, relative mycelial growth, and discriminatory dose. Fungicide-amended media was prepared by adding commercial-grade fungicides to ten-fold serial dilutions from an initial stock solution of 10 μg a.i. mL^{-1} of deionized sterile water to final concentrations of 10, 1, 0.1, 0.01 and 0.001 μg a.i. mL^{-1} PDA (Putman et al., 2010; Stephens and Kaminski, 2019) for all fungicides except for triadimefon. Based on a preliminary study, final concentrations for triadimefon were determined to be 100, 10, 1, 0.1, 0.01 μg a.i. mL^{-1} PDA. Isolates were screened against nine commercially available DMI fungicides including propiconazole (1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole) (Banner MAXX, Syngenta Crop Protection, Greensboro, NC), mefentrifluconazole (2*RS*)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1*H*-1,2,4-triazol-1-yl) propan-2-ol), (Maxtima, BASF corporation, Research Triangle Park, NC), myclobutanil (a-butyl-a-(chlorophenyl)-1H-1,2,4,triazole-1-propanenitrile) (Eagle, Dow AgroSciences LLC, Indianapolis, IN), flutriafol (α-(2-fluorophenyl)-α-(4-fluorophenyl)-1H-1,2,4-triazole-1-ethanol) (Rayora, FMC Corporation, Philadelphia, PA), triadimefon (1-(4-Chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanone) (Bayleton, Bayer Environmental Science, Research Triangle Park, NC), tebuconazole (α–[2-(4-chlorophenyl)ethyl]-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol) (Torque, NuFarm Americas Inc, Alsip, IL), metconazole (5-[(4-chlorophenyl)methyl]-2,2-dimethyl-1-(1 H-1,2,4-triazol-1-ylmethyl) cyclopentanol) (Tourney, Nufarm Americas Inc, Alsip, IL), and triticonazole (1,2-benzisothiazol-3(2H)-one) (Trinity, BASF, Research Triangle Park, NC).

The experiment was arranged as a completely randomized design with three replications and was repeated twice. Prior to initiation of the experiment, isolates were plated mycelium-side down on PDA for 4 to 5 days. An 8-mm diameter plug was removed from the margin of an actively growing colony and plated mycelium-side down on fungicide-amended PDA. Plates were sealed with parafilm and placed on the laboratory bench at 22 ± 2°C. After 48 h of growth, measurements of each culture were taken in two directions, perpendicular to each other, to determine isolate growth (Putman et al., 2010).

All data were used to determine RMG, EC_{50}, and ultimately a discriminatory dose for each fungicide. Relative mean growth, which included the growth of isolates on amended media relative to growth of the isolate on non-amended media, was determined in Microsoft Excel (Jo et al., 2006; Stephens Kaminski, 2019). Statistical analysis was carried out with the Probit procedure in SAS software (SAS Institute Inc., Cary, NC, USA) to determine the EC_{50} values for each fungicide. To determine discriminatory doses for each fungicide, RMG was regressed against the log EC_{50} values of all five doses evaluated for each fungicides using the PROC REG procedure in SAS. From the five dose concentrations evaluated, the dose that yielded the highest R^2 was determined to be the discriminatory dose for each fungicide.

Sensitivity screening

Six isolates of known levels of resistance to propiconazole were screened across the determined discriminatory doses of commercially available DMIs. These isolates had been collected from various locations in the Northeast United States (Stephens and Kaminski, 2019) (Table 3.2). Two of the isolates were collected from golf course tees at the Vineyard Golf Club located in Marth’s Vineyard, MA. These isolates had never been exposed to fungicides and served as the sensitive baseline population. Previous experiments found these two isolates to have an RMG of 31.25 when screened against propiconazole. Isolates collected from a golf course rough in Thendara, NY and a research fairway at the Joseph Valentine Turfgrass Research Center located in University Park, PA were considered moderately insensitive with RMGs of 56.12 and 55.22, respectively. Isolates considered highly insensitive to propiconazole, with RMGs of 92.01 and 92.97, had been collected from a golf course tee in Lake harmony, PA and a golf course fairway in Flourtown, PA, respectively (Stephens and Kaminski, 2019). Plugs of PDA with mycelia of the six isolates were obtained from permanent storage. Prior to use, isolates had been stored at 4°C in 2.0 mL microcentrifuge tubes containing 1 mL sterile de-ionized water. Plugs were grown on AWA for 5 d prior to transferring to PDA. Antibiotic water agar and PDA were prepared as previously described. Isolates were grown on PDA for 3 d. Eight-millimeter-diameter plugs of actively growing mycelium were transferred to PDA amended with fungicides at previously determined discriminatory dose concentrations of 1.0 μg a.i. mL^{-1} PDA for myclobutanil, triadimefon, mefentrifluconazole, metconazole and triticonazole. Discriminatory doses for other fungicides were 0.1 μg a.i. mL^{-1} PDA for flutriafol and tebuconazole and 0.01 μg a.i. mL^{-1} PDA propiconazole and prothioconazole.

Clarireedia speciation

Isolates utilized in this study were originally classified as *Sclerotinia homoeocarpa* and had not been previously speciated under the new genus *Clarireedia*. To ensure accurate assessment of the sensitivity of a known *Clarireedia* species, characterization of the isolates was determined using molecular analysis. The six previously mentioned isolates were grown on PDA for three days and plugs of actively growing mycelium were extracted. These plugs were then grown in potato dextrose broth (PDB) (Difco Laboratories, Detroit, MI) and set on a platform rotary shaker (Eppendorf, Enfield, CT) at 22 ± 2°C. Media was prepared by adding 12 g of PDB to 500 ml of water and mixing thoroughly prior to autoclaving for 25 min at 125°C. Media was allowed to cool to room temperature prior to adding PDA plugs of each isolate.

After 7 to 10 d, fungal mycelium mats of each isolate were separated from PDB using a vacuum attached to a sterile Buchner funnel lined with sterile filter paper. Collected mycelium was transferred to microcentrifuge tubes and lyophilized in a freeze dryer (Labconco Corporation, Kansas City, MO) set at -50^{o}C for 48 h. Lyophilized tissue was stored at -20^{o}C prior to DNA extraction. Extraction of fungal DNA was completed using the Qiagen DNA extraction kit as per the manufacturer’s protocol (Qiagen N.V., Hilden, Germany). Fungal mycelium was ground, lysed, and precipitated using the Qiagen DNeasy Plant tissue kit. Tubes containing precipitated DNA were centrifuged through the QIA shredder for 2 minutes at 14000 rpm to homogenize lysed tissue. A total of 450 uL of AW1 buffer was mixed with homogenized tissue, and then 650 uL of the mixture added to a spin column and centrifuged for 1 minute at 8000 rpm. This step was repeated twice. A 500 uL aliquot of AW2 buffer was then added and column centrifuged at 14000 rpm for 2 minutes. The spin column was transferred to a sterilized microcentrifuge tube, 50 uL of AE buffer added and incubated for 5 min at room temperature (22 ± 2^{o}C). This final solution was centrifuged for 1 min at 8000 rpm and the elution stored at -20^{o}C.

A nanodrop 1000 spectrophotometer was used to determine the purity and concentration of DNA material (Thermo Fischer Scientific, Waltham, MA). The primers ITS-4 and ITS-5 were used to amplify the ITS region based on previous studies (Salgado-Salazar et al., 2018). A master mix was created by combining 12.5 uL GoTaq G2 Green Master mix, 1.25 uL of each primer, and 9 uL of nuclease-free water in 1 mL centrifuge tubes. Tubes were vortexed for 10 sec and centrifuged for 10 sec prior to transferring to PCR tube strips. Previously extracted DNA (1 uL) was added to each reaction tube containing the master mix, vortexed for 10 sec, and placed in an Eppendorf Mastercycler Nexus Gradient (Eppendorf, Hamburg, Germany). The thermocycler raised the temperature to 95^{o}C and maintained it for a period of 4 min. A total of 35 cycles of denaturation at 95^{o}C for 30 sec, annealing at 55^{o}C for 30 sec, and elongation at 72^{o}C for 60 sec were then performed. Eventually, the thermocycler maintained the 72^{o}C for another 10 min before dropping to 4^{o}C.

Amplified PCR fragments were cleaned using the Wizard SV Gel and PCR clean up system as per the manufacturer's protocol (Promega Corporation, Madison, WI). An equal amount (25 uL) of membrane binding solution were added to the PCR product, transferred into an SV mini column and its collection tube, incubated at room temperature for 1 min, and centrifuged for another 1 min. Flow through solution was discarded, 700 uL of membrane wash solution (MWS) added and centrifuged again at 14000 RPM for 1 min. This step was repeated but with 500 uL of MWS and centrifuged for 5 min. The SV mini column was transferred to a new collection tube and 50 uL of nuclease-free water added. The contents were incubated at room temperature (22 ± 2^{o}C) for 1 min and centrifuged at 14000 rpm for 1 min. The SV mini column was discarded and flow through stored at 4^{o}C. Samples were Sanger sequenced at the Penn State Genomics Core Facility (University Park, PA). Produced sequences were manually trimmed and edited for low quality alignments. Both forward and reverse reads were created with Chromas Pro software (Informer Technologies, Inc, Los Angeles, CA).

Harmonized sequences were compared to sequences of the described species of *Clarireedia* genus within GenBank (NCBI, 2021) using basic local alignment search tool (BLASTn) for species level identification (Salgado-Salazar et al., 2018).

Results

EC$_{50}$, relative mean growth, and discriminatory doses

Values of EC$_{50}$ were determined for each isolate across the nine commercially available fungicides (Fig. 3.1) using the Probit procedure in SAS. Relative mean growth was determined for each isolate and then regressed against the log EC_{50} values (Fig 3.2). Out of five concentrations evaluated per fungicide, the concentration whose regression model produced the highest R^2 was considered the discriminatory dose (Putman et al., 2010; Stephens and Kaminski, 2019). After analysis of the fifty isolates, three discriminatory doses including 0.01 for propiconazole and prothioconazole, 0.1 for flutriafol and tebuconazole, and 1.0 μg a.i. mL^{-1} PDA for triadimefon, myclobutanil, metconazole, triticonazole, and mefentrifluconazole were identified. The nine fungicides had R^2 values ranging from 60.7% to 95.4% (Table 3.1).

Sensitivity screening

When screening six isolates for sensitivity (Table 3.2) across nine commercially available fungicides, a significant three-way interaction between experimental run, fungicide, and isolate sensitivity was observed (Table 3.3). The sensitive baseline population exhibited a wide range of variation in mycelial growth among the nine fungicides evaluated. Relative mean growth across sensitive isolates and all fungicides ranged from 0.0 to 62.5% and 0.0 to 58.7% for the first and second experimental run, respectively (Table 3.4). In both experimental runs, mefentrifluconazole and myclobutanil resulted in the least mycelial growth across isolates of all sensitivity. Triticonazole had similar RMG when screened across sensitive and moderately sensitive isolates. Prothioconazole and triadimefon generally resulted in the highest RMG across all isolates. When evaluated using moderately insensitive populations, RMG ranged from 15.7 to 59.1% and 5.6 to 59.3% across the first and second experimental run, respectively. There was a consistent increase in mycelial growth as the level of insensitivity went from moderately to highly insensitive populations.

Mefentrifluconazole completely inhibited mycelial growth of both sensitive isolates. Similar to mefentrifluconazole, myclobutanil and triticonazole had similar RMG when screened across sensitive isolates. Sensitive isolates had the highest RMG when grown in media amended with triadimefon and prothioconazole. None of the fungicides completely inhibited mycelial growth of the moderately and highly insensitive isolates. There were varying levels of mycelial growth suppression across the highly insensitive isolates, with prothioconazole having the least suppression of growth and mefentrifluconazole and myclobutanil providing the highest suppression. Although there were inconsistencies in mycelial growth suppression among fungicides against moderately insensitive isolates, prothioconazole resulted in the highest mycelial growth.

Clarireedia speciation

Speciation of the six isolates used in this study were determined via DNA amplification using ITS-4 and ITS-5 primers. A single isolate was determined to be contaminated during the PCR procedure and excluded from the study. Based on sequences obtained, five isolates were determined to be *C. jacksonii* when compared to sequences under accession numbers of described by Salgado Salazar et al. (2018) (Fig 3.3). There was a 99.83 to 100 % match between the sampled isolates and *C. jacksonii* (MF96320.1). When compared to *C. bennettii* (MF964321.1), *C. homoeocarpa* (MF964322.1) and *C. monteithiana* (KF545306.1), matches of 92.47, 92.81, and 97.60% were obtained, respectively.

Discussion

There is a wide variety of studies that have looked at the resistance and sensitivity levels of various *Clarireedia* spp. populations to propiconazole (Golembiewski, 1995; Jo et al., 2006; Koch et al., 2009; Putman et al., 2010; Stephens and Kaminski, 2019). However, despite the wide scope of DMI fungicides available for commercial use in the USA, the other eight chemistries have limited or no studies investigating development of *Clarireedia* spp. insensitivity. Isolates from the baseline population exhibited a varying response to all DMIs used in the study. Mefentrifluconazole completely suppressed mycelial growth of the sensitive isolates. When evaluated against moderately and highly sensitive populations, isolates screened against mefentrifluconazole were able to moderately grow. Suppression of mycelial growth in sensitive populations when screened against myclobutanil and triticonazole, which have been available for use in the turfgrass market for many years, was similar to mefentrifluconazole. This concurs with findings by Koch et al., (2009) indicating that previous fungicide applications, influence the levels of sensitivity to fungicides of the same class. Isolates collected from the golf course roughs appeared to be more sensitive to propiconazole than isolates from fairways and putting greens (Koch et al., 2009). Variability in mycelial growth within the sensitive population could be attributed to the inherent ability to reduce mycelial growth among DMI fungicides. There have been claims that recently introduced fungicides, such as mefentrifluconazole and flutriafol, offer enhanced control of insensitive *Clarireedia* spp. populations. Results from this trial indicate that fungicide suppression of mycelial growth in highly insensitive isolates is correlated to that of sensitive isolates, with only a relative increase in mycelial growth.

Differences among experimental runs in this study may be attributed to the fact that the experiments went over a period of approximately four months. When isolates were screened against propiconazole, they were found to have reduced RMG when compared to the initial screening by (Stephens and Kaminski, 2019). For instance, RMG of the highly insensitive isolates ranged from 50.4 to 71.7% which is lower compared to the initial RMGs (92.0 to 93.0%) screened against propiconazole (Stephens and Kaminski, 2019). This could be attributed to decrease of pathogen viability while in permanent storage. Some fungi have been found to undergo pleomorphism and physical alterations in fungal morphology has been observed when fungi are extracted from storage (Borman et al., 2006). This was also observed when determining discriminatory doses across the fifty isolates. In this study, the discriminatory dose for propiconazole was 0.01 μg a.i. mL^{-1} PDA, tenfold less than that found by (Jo et al., 2006). This can be explained by the observed reduction in mycelial growth of isolates due to long term storage. However, there was a < 5% difference in the R^2 between a concentration of 0.1 and 0.01 μg a.i. mL^{-1} PDA indicating that these differences were small and both doses may be effective when screening for resistance.

Discriminatory doses determined in this study can be used in the future to quickly screen for *Clarireedia* spp. resistance across the DMI fungicides. To our knowledge, there are no studies describing the sensitivity of *Clarireedia* spp. to all commercially available DMI fungicides. Sensitivity screening data suggests that cross resistance in mefentrifluconazole might be present. Isolates across all fungicides and both experimental runs were most sensitive to the recently introduced mefentrifluconazole. However, mycelial growth of moderately and highly insensitive isolates still occurred on mefentrifluconazole-amended media. This is in line with data from a study comparing the sensitivity of *Monilinia fruticola* (Wint.) to different DMI fungicides including tebuconazole, propiconazole, and mefentrifluconazole (Ishii et al., 2021). Sensitivity of the pathogen was higher when evaluated on mefentrifluconazole than when evaluated across the other three fungicides. However, on *Cercospora beticola*, *Alternaria* spp., and *Colletotrichum* spp., the inhibitory capacity of mefentrifluconazole was either equal to or slightly less than difenoconazole, tebuconazole, and propiconazole (Ishii et al., 2021). Cross resistance of *Clarireedia* spp. in mefentrifluconazole is yet to be reported. Results from this trial indicate that mefentrifluconazole works most effectively on sensitive isolates but still provided a relatively lower RMG when screened across moderately or highly insensitivity to other DMIs. Cross resistance in DMIs has been observed in other fungal pathogens such as *Podosphaera xanthii*, causal pathogen for cucumber powdery mildew, that are resistant to myclobutanil, triflumizole, difenoconazole, and mefentrifluconazole (Ishii et al., 2021). Although more studies are required to determine presence of resistance to mefentrifluconazole in *Clarireedia* spp, these results indicate that mefentrifluconazole is prone to have pathogens develop cross resistance when used over a period of time, but still may outperform other DMI fungicides for controlling dollar spot.

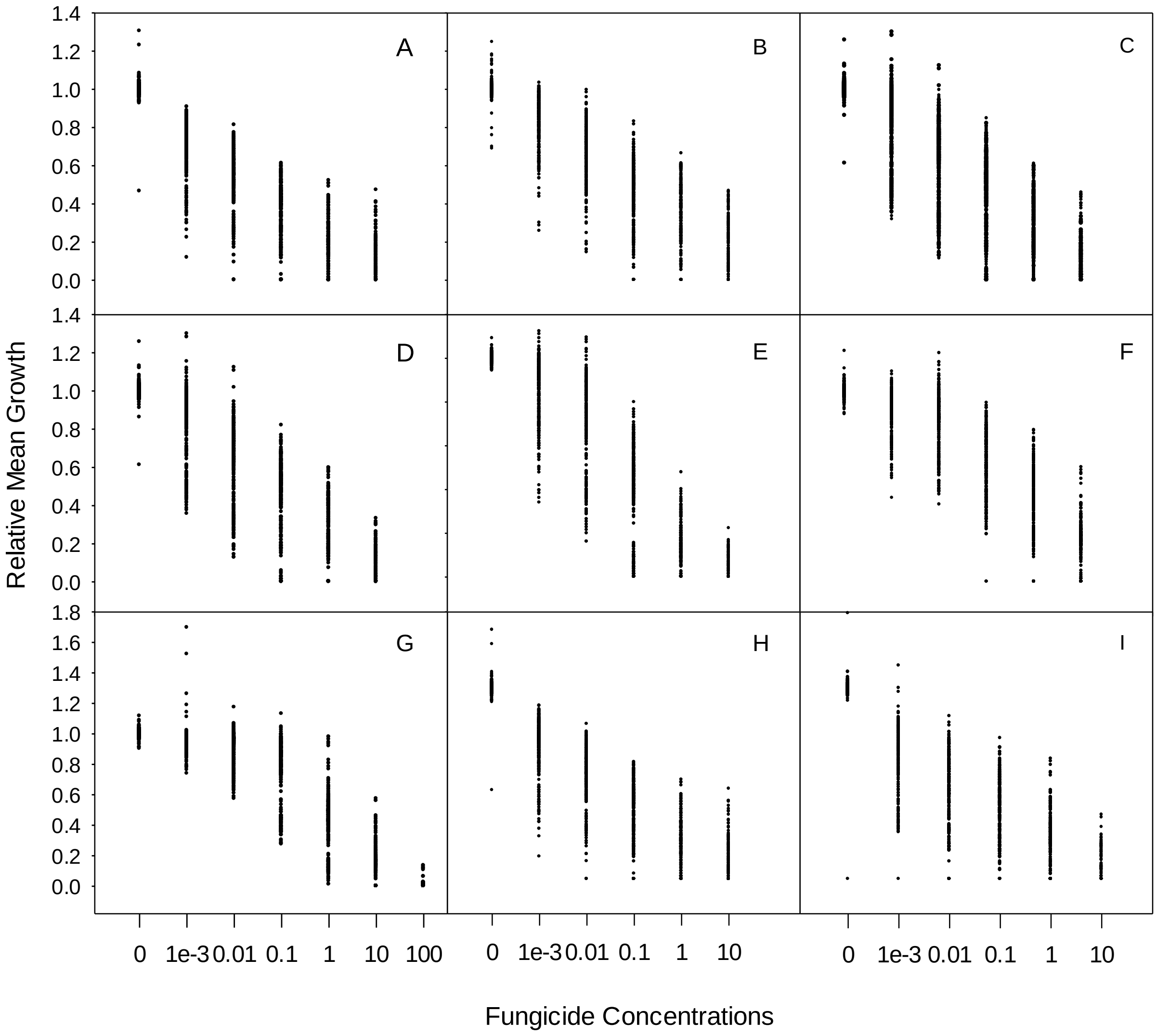

Figure 3.1. *Clarireedia* spp. mycelium growth across five concentrations of commercially available demethylation inhibitor fungicides. Letters indicate specific fungicides as follows: mefentrifluconazole (A), tebuconazole (B), flutriafol (C), myclobutanil (D), prothioconazole (E), metconazole (F), triadimefon (G), triticonazole (H), and propiconazole (I) .

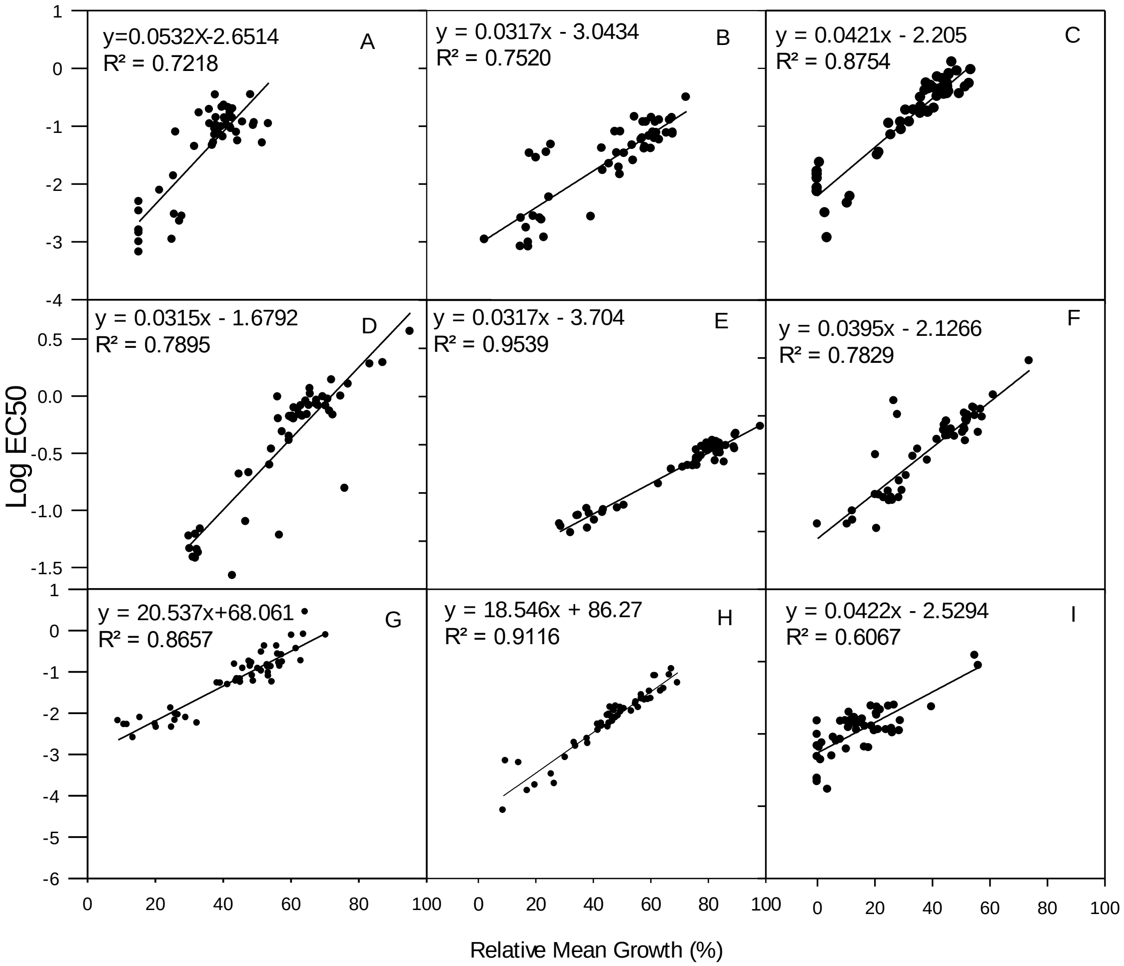

Figure 3.2. Relationship between LogEC$_{50}$ values and relative mycelial growth of nine commercially available demethylation inhibitor fungicides. Fungicides evaluated include myclobutanil (A), flutriafol (B), triticonazole (C), triadimefon (D), prothioconazole (E), metconazole (F), tebuconazole (G), propiconazole (H), and mefentrifluconazole (I).

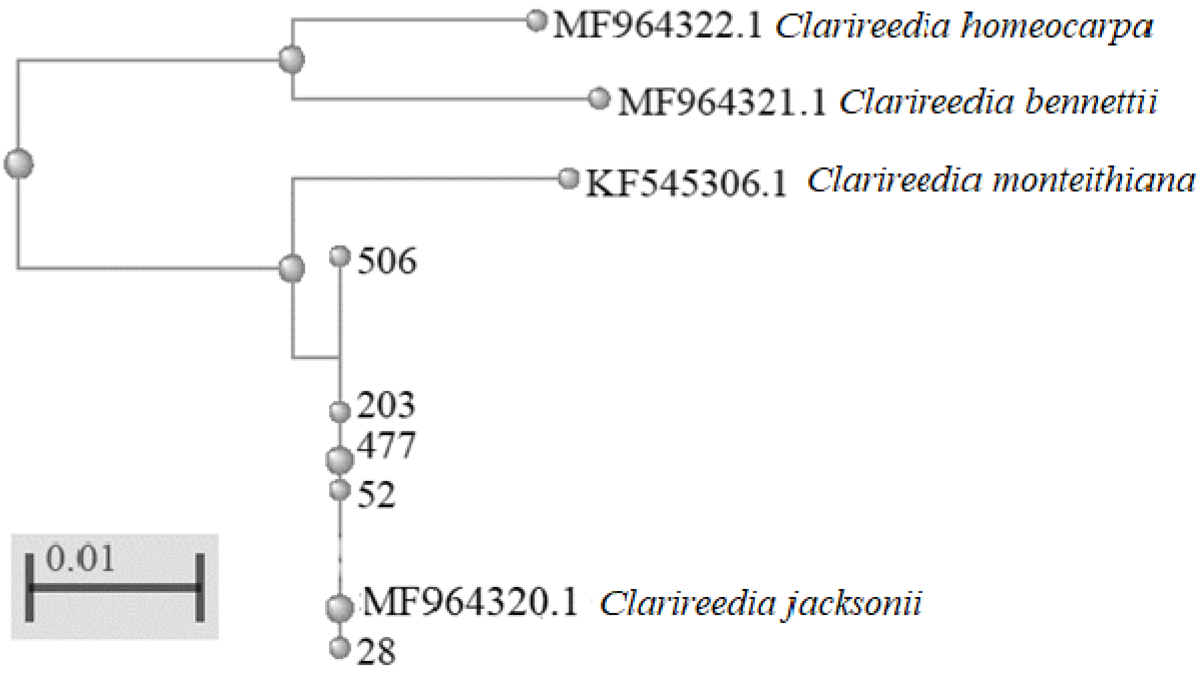

Figure 3.3. Neighbor joining tree showing the relationship between collected isolates and existing *Clarireedia* spp.

Table 3.1. Determined discriminatory doses for nine demethylation inhibitor fungicides.

Fungicide	Discriminatory dose[z] (µg a.i. mL^{-1} PDA)	R^2	P-Value
Propiconazole	0.01	0.7533[y]	<0.0001
Triadimefon	1.0	0.7895	<0.0001
Prothioconazole	0.01	0.9539	<0.0001
Myclobutanil	1.0	0.7218	<0.0001
Mefentrifluconazole	1.0	0.6067	<0.0001
Flutriafol	0.1	0.7520	<0.0001
Tebuconazole	0.1	0.8657	<0.0001
Metconazole	1.0	0.7829	<0.0001
Triticonazole	1.0	0.8754	<0.0001

[z] Discriminatory doses for commercially available DMI fungicides were determined by regressing the log EC_{50} against the relative mean growth. Out of the five evaluated doses, the one whose regression yielded the highest R^2 was considered the discriminatory dose.

Table 3.2. Isolates used to screen for sensitivity across demethylation inhibiting fungicides.

Isolate	Resistance level	Place of origin	RMG[z]	Grass species
52	Sensitive[y]	Edgartown MA	31.25[y]	*Agrostis stolonifera*
203	Sensitive	Edgartown, MA.	31.25	*A. stolonifera*
28	Moderately insensitive	Thendara, NY	56.12	*Poa pratensis*
477	Moderately insensitive	University Park, PA	55.22	*A. stolonifera*
506	Highly insensitive	Lake Harmony, PA	92.01	*A. stolonifera*
807	Highly insensitive	Flourtown, PA	92.97	*A. stolonifera*

[z] Relative mean growth (RMG) of isolates previously screened against propiconazole.

[y] Sensitive isolates were collected from Vineyard Golf Club and had never been treated with fungicides.

Table 3.3. Analysis of variance for relative mean growth of isolates of *Clarireedia jacksonii* previously expressing varying levels of sensitivity to propiconazole when screened against 9 commercially available fungicides.

Source of Variation	P-Value
Fungicide (F)	<0.0001
Resistance level (RL)	<0.0001
Experimental run (ER)	0.1167
(RL)*ER)	0.7879
(F)*(RL)	0.0253
(F)*(ER)	0.0176
(F)*(ER)*(RL)	0.0230

Table 3.4. Relative mean growth of isolates of *Clarireedia jacksonii* previously expressing varying levels of sensitivity to propiconazole when screened against nine commercially available fungicides.

	Experimental Run 1			Experimental Run 2		
Fungicide[z]	Sensitive[y]	Moderately Sensitive[x]	Highly Insensitive[w]	Sensitive	Moderately Sensitive	Highly Insensitive
Propiconazole	35.2 cd	41.7 ab	50.4 c	21.3 cd	40.2 ab	71.7 ab
Triadimefon	62.5 a	50.7 a	85.0 a	51.5 ab	48.8 ab	45.2 de
Prothioconazole	49.7 ab	59.1 a	76.4 ab	58.7 a	59.3 a	84.7 a
Myclobutanil	0.0 g	19.7 cd	40.5 cd	7.2 de	10.3 cd	34.9 ef
Mefentrifluconazole	0.0 g	15.7 d	23.6 e	0.0 e	5.6 d	22.0 f
Flutriafol	23.8 de	49.5 a	68.6 b	19.9 cde	34.8 abc	48.5 cde
Tebuconazole	47.1 bc	40.7 ab	81.2 a	32.6 bc	57.9 a	60.5 bc
Metconazole	15.4 ef	39.7 abc	49.5 c	20.2 cde	44.7 ab	54.2 cd
Triticonazole	4.4 fg	26.1 bcd	37.7 d	9.5 de	23.1 bcd	50.2 cd

[z] Commercially available demethylation inhibitor fungicides.

[y] Sensitive populations had predetermined RMGs of 31.25 against propiconazole in a previous study (Stephens and Kaminski, 2019).

[x] Moderately sensitive isolates had predetermined RMGs of 55.22 and 56.12 against propiconazole in a previous study (Stephens and Kaminski, 2019).

[w] Highly sensitive populations had predetermined RMGs of 92.01 and 92.97 against propiconazole in a previous study (Stephens and Kaminski, 2019).

APPENDIX. VARIABLE DOLLAR SPOT CONTROL AMONG COMMERCIALLY AVAILABLE DEMETHYLATION-INHIBITING FUNGICIDES.

Introduction

Dollar spot, caused by one of five species within the genus *Clarireedia*, is one of the most common diseases in amenity turfgrass systems around the world (Salgado-Salazar et al., 2018; Hu et al., 2019). Of the five described species, *C. jacksonii* C. Salgado, L.A. Beirn, B.B. Clarke, and J.A. Crouch has been described as the causal pathogen of dollar spot in cool-season grasses in the United States (Salgado-Salazar et al., 2018). Although cultural practices can reduce disease severity, they generally do not provide complete or acceptable suppression of disease symptoms. The use of fungicides, therefore, is often required to meet low disease thresholds of highly maintained turfgrass systems. Repeated use of fungicides for dollar spot control, however, has led to the development of insensitive populations of *Clarireedia* spp.

Fungicide resistance, or insensitivity, can be categorized into either quantitative or qualitative resistance. Quantitative resistance occurs where a fungicide's efficacy against a pathogen population is reduced relative to historical suppression. In these cases, disease suppression may still be achieved, but the duration of control reduced. Qualitative resistance is observed when a fungicide that previously controlled a pathogen population lacks efficacy against the same population. To overcome quantitative resistance, turfgrass managers may increase the rate of fungicide applied or reduce the application intervals. On the other hand, fungicide exhibiting qualitative resistance results in their complete ineffectiveness. Resistance development poses a threat to the existing fungicide classes as it can lead to the loss of active ingredients available for management, thus leaving turfgrass managers with fewer chemical control options.

Clarireedia spp. have been shown to be resistant or have reduced sensitivity to various chemical classes that are registered for use in turfgrass. Resistance has been reported in four commercially available fungicide classes including the dicarboximides, benzimidazoles, demethylation inhibitors (DMIs), and most recently in succinate dehydrogenase inhibitors (SDHIs) (Warren et al., 1974; Detweiler et al., 1983; Golembiewski et al., 1995; Popko et al., 2018).

Resistance to benzimidazoles was first reported in the United States across various golf courses in Ohio, New Jersey, Pennsylvania, and Illinois (Warren et al., 1974). Where resistance to thiophanate-methyl exists, the fungicide is no longer a viable option for dollar spot control (Jo et al., 2006; Putman et al., 2010). Development of field resistance to dicarboximides was first reported in 1983 (Detweiler et al., 1983), but appears to be sporadic with reduced sensitivity diminishing from a population where it was previously reported (Jo et al., 2006; Putman et al., 2010).

The broad spectrum DMIs developed field resistance more than 10 years after their release to the turfgrass market. Subsequent studies have confirmed reduced sensitivity to the DMIs throughout various regions of the United States and Canada where cool-season turfgrasses are commonly grown. Analysis of the fungicide application frequency can partially be used to determine pathogen resistance to DMIs. A study carried out by Hsiang et al. (1997) in Canada investigated fungicide sensitivity in pathogen populations prior to any DMI fungicide class registrations in the country. Results indicated a wide variability in sensitivity across all isolates screened. However, out of eight sampled populations, one population exhibited reduced sensitivity to propiconazole. This single population had been collected from a location near the US/Canada border and was suspected to have been exposed to the fungicide class. A follow up study conducted a decade later on the same pathogen populations found that isolates exposed to DMIs over the ten years had a reduced level of sensitivity to propiconazole (Hsiang et al., 2006). Cross resistance, or resistance across multiple active ingredients with a single chemical class, has also been documented (Vargas et al., 1992; Golembiewski et al., 1995).

In addition to cross resistance within individual chemical classes, isolates collected in the Northeastern United States from different locations exhibited reduced fungicide sensitivity to multiple chemical classes including active ingredients within the DMIs, dicarboximides, benzimidazoles, and SDHIs (Stephens and Kaminski, 2019). This represents suspected resistance or insensitivity across four fungicide classes registered for us for the control of *Clarireedia* spp. (Jo et al., 2008; Putman et al., 2010; Stephens and Kaminski, 2019). There are ten DMI fungicides registered for use in turf with a total of nine that are commercially available as stand-alone products.

Variation in efficacy of the commercially available DMI fungicides and differences in control of populations with varying sensitivities, however, remains relatively unknown. Additionally, previous studies have focused on propiconazole when investigating reduced field sensitivity within the fungicide group. Therefore, the objectives of this study were to: 1) evaluate the variation in dollar spot control among commercially available DMI fungicides and 2) compare the effect of commercially DMI fungicide application intervals and rates for dollar spot suppression.

Materials and Methods

In 2020, two field trials were initiated at the Joseph Valentine Turfgrass Research Center (Valentine) and Center Hills Country Club located in University Park and State College, PA, respectively. The Valentine site was a 17-year-old mixed stand of ‘Penneagle’ creeping bentgrass (*Agrostis stolonifera* L.) consistently used as a research fairway. Turfgrass at Centre Hills consisted of a mature stand of ‘Pennlinks’ creeping bentgrass. Both sites were mowed three times per week to a height of 12.3 mm and irrigated as necessary to prevent wilt. Soil at Valentine site was a Hagerstown silt loam with a pH of 6.6 and organic matter content 5.25%. Soil at Centre Hills Country Club was a Hagerstown silty clay loam with a pH of 6.4 and organic matter content of 7.0%. All trials were repeated in 2021 and an additional location in State College added. In 2021, Mountain View Country Club (Mountain View), located in Boalsburg, PA, was included in the study due to previous research indicating reduced sensitivity to propiconazole (Stephens and Kaminski, 2019). The site was approximately a 10-90% mixed stand of perennial ryegrass (*Lolium perenne* L.) and an unknown cultivar of creeping bentgrass. Soil at the Mountain View was an Opequon Hagerstown with a pH of 6.5 and organic matter content of 6.7%. Turfgrass at Mountain View was mowed at 12.7 mm and irrigated to prevent wilt.

Treatments included ten fungicides and 2 fungicide rates. Fungicide treatments included the following demethylation-inhibiting fungicides: propiconazole (1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole) (Banner MAXX, Syngenta Crop Protection, Greensboro, NC), mefentrifluconazole (2*RS*)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1*H*-1,2,4-triazol-1-yl) propan-2-ol, (Maxtima, BASF corporation, Research Triangle Park NC), myclobutanil (a-butyl-a-(chlorophenyl)-1H-1,2,4,triazole-1-propanenitrile) (Eagle, Dow AgroSciences LLC, Indianapolis, IN), flutriafol (α-(2-fluorophenyl)-α-(4-fluorophenyl)-1H-1,2,4-triazole-1-ethanol) (Rayora, FMC Corporation, Philadelphia, PA), triadimefon 1-(4-Chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanone (Bayleton, Bayer Environmental Science, Research Triangle Park, NC), tebuconazole (α–[2-(4-chlorophenyl)ethyl]-

α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol) (Torque, Cleary Chemical LLC, Dayton, NJ,), metconazole (5-[(4-chlorophenyl)methyl]-2,2-dimethyl-1-(1 H-1,2,4-triazol-1-ylmethyl) cyclopentanol) (Tourney, NuFarm Americas Inc, Alsip, IL), and triticonazole (1,2-benzisothiazol-3(2H)-one) (Trinity, BASF, Research Triangle Park, NC). Pydiflumetofen, 1H-Pyrazole-4-carboxamide,3-(difluoromethyl)-N-methoxy-1-methyl-N-[1-methyl-2-(2,4,6 trichlorophenyl) ethyl] (Posterity, Syngenta Crop Protection, Greensboro, NC) and a nontreated control were also included. Fungicides were applied on either a 14- or 21-d interval based on low and high label rates of each fungicide, respectively. All fungicide treatments, rates, and application intervals are shown in Table 1.

All treatments were applied using a CO_2 pressurized handheld sprayer fitted with an air induction flat fan nozzle (AI9504EVS) calibrated to deliver 409 L ha^{-1} at 276 kPa. Plots measured 0.9 m x 1.8 m and were arranged as a randomized complete block design with four replications. All treatments were initiated on 24 and 15 June and reapplied every 14 or 21 days based on fungicide application rates in 2020 and 2021, respectively. Dollar spot infection centers (DSIC) were counted weekly as a measurement of disease severity. Quality was visually rated on a 1 to 9 scale, where 1 = entire plot brown or dead, 6 = minimum level of acceptable fairway turfgrass quality, and 9 = optimum greenness and density. Data were subjected to the MIXED procedure in SAS (SAS Institute Inc., Cary, NC, USA) and means separated using Fisher's Protected least significant difference test at $P \leq 0.05$.

Results

In 2020, dollar spot disease pressure was generally low around State College area. Analysis of variance from both sites did not reveal any significance of the main effects of fungicide or application interval. The data revealed no significant differences between treatments at either location. In 2021, disease severity was considered moderate to severe with an average of 139, 1 and 220 DSIC during peak disease activity at Valentine, Centre Hills and Mountain View, respectively.

Valentine Turfgrass Research facility

In 2021 data analysis indicated statistical differences amongst the fungicide treatments. Across all rating dates, there was one date with a significant treatment and application interval interaction This can however be attributed to dollar spot epidemics occurring right before the scheduled application date for the 14-day application interval, and just seven days after the 21-day applications had been made. Mefentrifluconazole, prothioconazole and pydiflumetofen, an SDHI consistently had the least amount of dollar spot throughout the season.

Mountain View Country Club

On a 14-day application interval regime, prothioconazole and mefentrifluconazole resulted in the least number of spots within the plots. However, 21 days after the final application, the amount of disease spots on the plots treated with triticonazole and propiconazole were not significantly different from the nontreated control plots. Pydiflumetofen resulted in the least amount of disease spots on the treated plots 21-days after the last application. On 21-day application interval regime, significant differences were observed one month after the initial treatment. Tebuconazole, propiconazole, prothioconazole and triadimefon resulted in the least count of dollar spot infection centers. Myclobutanil and metconazole, although significantly different from the control plots, had the most count of DSIC when compared to other treatments five weeks after initial application. Seven days after the fourth and final application, propiconazole resulted in the least amount of

DSIC, while myclobutanil resulted in the highest number of DSIC second after the nontreated control plots.

Centre Hills Country Club

The trial at this site did not get any disease epidemics, hence the data from that trial was not analyzed.

Discussion

Table 4.1. Demethylation inhibitor fungicides and fungicide rates evaluated for the suppression of dollar spot on creeping bentgrass fairways at Valentine Turfgrass Research Facility, Centre Hills Country Club, and Mountain View Country Club in 2020 and 2021.

	Application rate (L ha^{-1})	
Active ingredient	14-d interval[z]	21-d interval[y]
Mefentrifluconazole	0.64	1.28
Myclobutanil	3.18	7.64
Metconazole	0.57	1.18
Tebuconazole	1.91	3.50
Propiconazole	1.59	6.36
Triadimefon	1.59	3.18
Triticonazole	3.18	6.36
Flutriafol	2.23	4.46
Pydiflumetofen	0.26	0.51
Prothioconazole[x]	0.60	0.60

[z] Fungicide treatments applied on a 14-day interval were applied on: 24 Jun, 8 Jul, 23 Jul, 5 Aug, 19 Aug, and 3 Sep in 2020; 26 May, 9 Jun, 21 Jul, 4 Aug in 2021.

[y] Fungicide treatments applied on a 21-day interval were applied on: 24 Jun, 15 Jul, 5 Aug, and 26 Aug 2020 and 26 May, 16 Jun, 7 Jul, and 4 Aug in 2021.

[x] Prothioconazole was only included in the 2021 study when it became commercially available following the registration of the fungicide for turfgrass use in the United States.

Table 4.2: Analysis of Variance for dollar spot severity on a creeping bentgrass fairway following applications of various demethylation inhibiting fungicides and pydiflumetofen at the Valentine Turfgrass Research Facility, 2020.

	Dollar spot infection centers[z]		
Treatment[y]	8 Jul	29 Jul	20 Aug
Fungicide			
Propiconazole	-	-	-
Triadimefon	-	-	-
Prothioconazole	-	-	-
Myclobutanil	-	-	-
Mefentrifluconazole	-	-	-
Pydiflumetofen	-	-	-
Flutriafol	-	-	-
Tebuconazole	-	-	-
Metconazole	-	-	-
Triticonazole	-	-	-
Nontreated	-	-	-
Application interval			
14-day	-	-	-
21-day	-	-	-
Source of variation			
Fungicide (F)	0.4507	0.4507	0.4507
Application interval (A)	0.3215	0.3215	0.3215
F*A	0.4507	0.4507	0.4507

[z] Dollar spot severity was assessed by counting the number of dollar spot infection centers within each plot.

[y] Treatments were applied on 24 Jun, 8 Jul, 15 Jul, 23 Jul, 5 Aug, 19 Aug, 26 Aug, and 3 Sep in 2020.

Table 4.3. Dollar spot severity on a creeping bentgrass fairway following applications of various demethylation inhibiting fungicides and pydiflumetofen at the Valentine Turfgrass Research Facility, 2021.

	Dollar spot infection centers (DSIC)[z]									
Treatment[y]	9 Jun	16 Jun	23 Jun	30 Jun	7 Jul	15 Jul	21 Jul	28 Jul	4 Aug	11 Aug
Fungicide										
Propiconazole	0 c[x]	3 cd	5 cd	3 de	6 de	9 c-f	19 de	14cd	23 cd	17 c
Triadimefon	1 c	6 bc	10 bc	9 bc	14 bc	18 cd	33 bcd	25 bc	24 cd	24 bc
Prothioconazole	0 c	4 bcd	2 cd	1 e	5 e	5 ef	13 ef	11 cd	9 cd	11 cd
Myclobutanil	0 c	5 bcd	7 bcd	6 cde	7 cde	17 cde	24 cde	17 bc	33 c	39 b
Mefentrifluconazole	0 c	0 d	0 d	0 e	1 e	0 f	1 f	1 d	1 d	0 d
Pydiflumetofen	0 c	0 d	1 d	1 e	0 e	0 f	0 f	1 d	0 d	0 d
Flutriafol	3 ab	9 b	14 ab	12 b	15 b	36 b	41 b	32 b	61 b	36 b
Tebuconazole	1 c	5 bcd	4 cd	2 e	5 de	9 def	18 de	17 bc	13 cd	17 c
Metconazole	0 c	4 bcd	6 cd	3 de	7 cde	14 cde	28 b-e	22 bc	18 cd	34 b
Triticonazole	2 bc	6 bc	9 bc	9 bcd	13 bcd	22 bc	39 bc	25 bc	32 c	33 b
Nontreated	4 a	17 a	23 a	27 a	30 a	80 a	113 a	92 a	123 a	165 a
Application interval										
14 days	-	-	-	-	-	-	-	-	41 a	-
21 days	-	-	-	-	-	-	-	-	20 b	-
Source of variation										
Fungicide (F)	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Application interval (A)	0.1432	0.2782	0.2505	0.0863	0.4358	0.2422	0.2985	0.2659	0.0143	0.4516
F*A	0.7966	0.1041	0.2850	0.1441	0.2368	0.5854	0.2168	0.2966	0.2873	0.0806

[z] Dollar spot severity was assessed by counting the number of dollar spot infection centers within each plot.

[y] Treatments were applied on the following dates: 26 May, 9 Jun, 21 Jul, 4 Aug, and on 26 May, 23 Jun, 4 Aug for 14-day and 21-day intervals respectively.

[x] Means followed by similar letters within each date and for each treatment are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

Table 4.4. Dollar spot severity on a creeping bentgrass fairway following applications of various demethylation inhibiting fungicides and pydiflumetofen at Mountain View Country Club, 2021.

	Dollar spot infection centers (DSIC) [z]							
Treatment[z]	**23 Jun**	**30 Jun**	**7 Jul**	**14 Jul**	**21 Jul**	**28 Jul**	**4 Aug**	**25 Aug**
Fungicide								
Propiconazole				22 b-e		5 d		
Triadimefon				23 b-e		13 cd		
Prothioconazole				21 cde		15 cd		
Myclobutanil				41 bc		20 bcd		
Mefentrifluconazole				0 e		16 cd		
Pydiflumetofen				5 de		11 cd		
Flutriafol				29 bcd		20 bcd		
Tebuconazole				28 bcd		20 bcd		
Metconazole				40 bc		24 bc		
Triticonazole				47 b		33 b		
Nontreated				114 a		59 a		
Source of variation								
Fungicide (F)	<0.0001	<0.0001	<0.0001	<0.0001	0.0013	0.0002	<0.0001	0.0039
Application interval (A)	0.0220	0.0025	0.0372	0.1554	0.2212	0.0002	<0.0001	0.9012
F*A	0.0303	0.0360	0.0044	0.0582	0.0177	0.0866	0.0002	0.0422

[z] Dollar spot severity was assessed by counting the number of dollar spot infection centers within each plot.

[y] Treatments were applied on the following dates: 26 May, 9 Jun, 23 Jun, 7 Jul, 21 Jul, and 4 Aug.

[x] Means followed by similar letters within each date and for each treatment are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

Table 4.5. Dollar spot severity on a creeping bentgrass fairway following applications of various demethylation inhibiting fungicides and pydiflumetofen on a 14-day interval at Mountain View Country Club, 2021.

	Dollar spot infection centers (DSIC)[z]					
Treatment[y]	23 Jun	30 Jun	7 Jul	21 Jul	4 Aug	25 Aug
Fungicide						
Propiconazole	43 bcd[x]	27 bcd	34 bcd	92 a-d	125 bc	237 a
Triadimefon	61 bc	29 bc	41 bc	112 ab	91 bcd	189 abc
Prothioconazole	3 e	2 e	0 e	42 cd	22 f	133 bc
Myclobutanil	45 bcd	12 cde	17 cde	103 abc	112 bcd	215 ab
Mefentrifluconazole	3 e	0 e	0 e	34 d	17 f	141 bc
Pydiflumetofen	34 cd	15 cde	9 de	67 bcd	24 ef	104 c
Flutriafol	46 bcd	13 cde	23 cde	103 abc	63 def	209 ab
Tebuconazole	25 de	6. de	17 cde	86 a-d	80 cde	214 ab
Metconazole	32 cde	12 cde	14 cde	87 a-d	88 bcd	232 ab
Triticonazole	68 b	39 b	51 ab	142 a	142 b	262 a
Nontreated	102 a	71 a	71 a	147 a	244 a	265 a
Source of variation						
Fungicide (F)	<0.0001	<0.0001	<0.0001	0.0013	<0.0001	0.0039
Application interval (A)	0.0220	0.0025	0.0372	0.2212	<0.0001	0.9012
F*A	0.0303	0.0360	0.0044	0.0177	0.0002	0.0422

[z] Dollar spot severity was assessed by counting the number of dollar spot infection centers within each plot.

[y] Treatments were applied on the following dates: 26 May, 9 Jun, 23 Jun, 7 Jul, 21 Jul, and 4 Aug.

[x] Means followed by similar letters within each date and for each treatment are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

Table 4.6. Dollar spot severity on a creeping bentgrass fairway following applications of various demethylation inhibiting fungicides and pydiflumetofen on a 21-day interval at Mountain View Country Club, 2021.

	Dollar spot infection centers[z]				
Fungicide[z]	23 Jun	30 Jun	7 Jul	21 Jul	4 Aug
Propiconazole	13 c[x]	3 c	7 c	43 de	1 e
Triadimefon	16 c	4 c	4 c	52 cde	18 cde
Prothioconazole	12 c	2 c	7 c	107 abc	8 de
Myclobutanil	53 b	18 b	37 b	96 abc	55 b
Mefentrifluconazole	25 bc	3 c	7 c	69 cde	32 bcd
Pydiflumetofen	21 c	5 c	3 c	31 e	4 de
Flutriafol	30 bc	7 c	20 bc	87 b-e	38 bc
Tebuconazole	19 c	5 c	15 c	70 cde	11 cde
Metconazole	31 bc	11 bc	19 c	134 ab	40 bc
Triticonazole	29 bc	9 c	8 c	53 cde	18 cde
Nontreated	102 a	71 a	72 a	147 a	244 a

[z] Dollar spot severity was assessed by counting the number of dollar spot infection centers within each plot.

[y] Treatments were applied on the following dates: 26 May, 16 Jun, 7 Jul, and 28 Jul.

[x] Means followed by similar letters within each date and for each treatment are not significantly different at $P \leq 0.05$ according to Fisher’s protected least significant difference test.

Table 4.7. Quality of a creeping bentgrass fairway following applications of various demethylation inhibiting fungicides and pydiflumetofen at the Valentine Turfgrass Research Facility, 2021.

	Turfgrass quality[z]							
Treatment[y]	16 Jun	23 Jun	7 Jul	15 Jul	21 Jul	28 Jul	4 Aug	11 Aug
Fungicide								
Propiconazole	8.0 ab[x]	7.8 bc	8.5 ab	7.8 ab	7.4 ab	7.8 ab	7.3 de	7.3 bc
Triadimefon	7.9 ab	7.3 de	7.6 de	6.8 de	6.8 bc	7.1 bcd	7.4 cde	6.9 cde
Prothioconazole	7.9 ab	8.0 ab	8.3 abc	7.9 ab	7.1 bc	7.6 abc	7.9 abc	7.8 ab
Myclobutanil	7.9 ab	7.4 cde	7.8 abc	7.3 bcd	6.5 c	7.1 bcd	7.1 de	6.3 f
Mefentrifluconazole	8.1 a	8.3 a	8.8 a	8.0 a	8.0 a	8.0 a	8.3 a	8.0 a
Pydiflumetofen	8.0 ab	8.3 a	8.5 ab	8.1 a	8.0 a	7.6 abc	8.0 ab	8.0 a
Flutriafol	7.9 ab	7.4 cde	7.8 cde	6.4 e	6.6 c	6.8 d	6.9 e	6.4 ef
Tebuconazole	7.8 ab	7.6 bcd	8.4 ab	7.6 abc	7.0 bc	7.3 bcd	7.5 bcd	7.4 bc
Metconazole	7.6 bc	7.9 ab	8.1 bcd	7.5 abc	6.9 bc	7.4 a-d	7.5 bcd	7.0 cd
Triticonazole	7.8 ab	7.4 cde	8.3 abc	7.0 cde	6.8 bc	7.0 cd	7.1 de	6.5 def
Nontreated	7.3 c	7.0 e	7.3 e	5.5 f	5.2 d	5.5 e	5.5 f	4.3 g
Application interval								
14 days	-	-	-	-	-	-	6.3 b	6.9 b
21 days	-	-	-	-	-	-	7.1 a	7.4 a
Source of variation								
Fungicide (F)	0.0288	<0.0001	<0.0001	<.0001	<0.0001	<0.0001	<0.0001	<0.0001
Application interval (A)	0.6326	0.2061	0.1933	0.1036	0.3002	0.1275	<0.0001	0.0072
F*A	0.5694	0.6509	0.3174	0.6224	0.4563	0.2964	0.0164	0.2492

[z] Turfgrass quality was visually assessed on a 0 to 9 scale, where 1 = entire plot brown or dead, 6 = minimum level of acceptable turfgrass quality and 9 = optimum greenness and density for a golf course fairway.

[y] Treatments were applied on the following dates: 26 May, 9 Jun, 21 Jul, 4 Aug, and on 26 May, 23 Jun, 4 Aug for 14-day and 21-day intervals respectively. 26 May, 9 Jun, 23 Jun, 7 Jul, 21 Jul, and 4 Aug.

.[x] Means followed by similar letters within each date and for each treatment are not significantly different at $P \leq 0.05$ according to Fisher’s protected least significant difference test.

Table 4.8. Quality of a creeping bentgrass fairway following applications of various demethylation inhibiting fungicides and pydiflumetofen at the Mountain View, 2021 on a 14-day interval.

	Turfgrass quality[z]					
Treatment[y]	23 Jun	30 Jun	15 Jul	21 Jul	28 Jul	4 Aug
Fungicide						
Propiconazole	6.3 cdx	6.3 de	6.8 a-d	5.5 cd	6.8 abc	6.0 bc
Triadimefon	6.0 cd	6.5 cde	6.5 bcd	5.5 cd	7.0 ab	6.3 b
Prothioconazole	7.5 ab	8.3 a	8.0 a	7.0 ab	7.8 a	7.5 a
Myclobutanil	6.8 bc	7.5 abc	7.0 abc	5.8 bcd	7.3 ab	5.8 bc
Mefentrifluconazole	8.3 a	8.3 a	8.0 a	7.5 a	7.8 a	7.8 a
Pydiflumetofen	6.0 cd	7.0 bcd	7.8 ab	6.0 bcd	7.3 ab	7.5 a
Flutriafol	5.8 de	6.8 b-e	6.8 ab	5.3 d	7.5 a	6.0 bc
Tebuconazole	7.0 bc	7.3 a-d	6.3 a-d	5.8 bcd	7.3 ab	6.0 bc
Metconazole	6.5 bcd	7.8 ab	7.3 abc	6.8 abc	7.3 ab	6.3 b
Triticonazole	4.8 ef	5.8 e	5.5 de	5.3 d	6.3 bc	5.3 cd
Nontreated	3.8 f	5.8 e	5.0 e	5.3 d	5.8 c	4.8 d
Source of variation						
Fungicide (F)	<0.0001	<0.0001	<0.0001	0.0844	0.0002	<0.0001
Application interval (A)	0.1812	0.0635	0.2539	0.3083	0.3550	<0.0001
F*A	0.0012	0.0075	0.0186	0.0007	0.0385	0.0075

[z] Turfgrass quality was visually assessed on a 1 to 9 scale where 6 = minimum level of acceptable turfgrass quality and 9 = perfect quality for a golf course fairway.

[y] Treatments were applied on the following dates: 26 May, 9 Jun, 23 Jun, 7 Jul, 21 Jul, and 4 Aug.

[x] Means followed by similar letters within each date and for each treatment are not significantly different at $P \leq 0.05$ according to Fisher’s protected least significant difference test.

Table 4.9. Quality of a creeping bentgrass fairway following applications of various demethylation inhibiting fungicides and pydiflumetofen on a 21-day interval at the Mountain View Golf Club, 2021.

	Turfgrass quality[z]					
Treatment[y]	23 Jun	30 Jun	15 Jul	21 Jul	28 Jul	4 Aug
Fungicide						
Propiconazole	7.8 a[x]	7.3 ab	8.0 a	7.0 ab	7.5 a	8.0 a
Triadimefon	7.0 ab	8.0 a	7.8 ab	6.8 abc	7.3 a	7.5 abc
Prothioconazole	7.3 a	7.5 ab	6.5 bc	5.5 bcd	7.0 a	7.8 ab
Myclobutanil	5.8 bc	7.0 b	6.8 abc	5.8 bcd	6.8 ab	6.8 cd
Mefentrifluconazole	6.8 abc	7.5 ab	8.0 a	5.8 cd	6.8 ab	7.3 a-d
Pydiflumetofen	6.5 abc	7.8 ab	8.0 a	8.0 a	7.5 a	8.0 a
Flutriafol	6.5 abc	7.5 ab	7.0 abc	6.5 abc	7.5 a	6.5 d
Tebuconazole	7.5 a	7.8 ab	7.0 abc	6.8 abc	7.0 a	7.5 abc
Metconazole	5.5 c	7.0 b	6.0 cd	4.8 d	6.0 bc	7.0 bcd
Triticonazole	7.0 ab	7.5 ab	7.3 abc	6.0 bcd	7.3 a	7.0 bcd
Nontreated	3.8 d	5.8 c	5.0 d	5.3 cd	5.8 c	4.8 e

[z] Turfgrass quality was visually assessed on a 1 to 9 scale where 6 = minimum level of acceptable turfgrass quality and 9 = perfect quality for a golf course fairway each plot.

[y] Treatments were applied on the following dates: 26 May,16 Jun, 7 Jul, and 28 Jul.

[x] Means followed by similar letters within each date and for each treatment are not significantly different at $P \leq 0.05$ according to Fisher’s protected least significant difference test.

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