Improved Strategies for Dollar Spot Suppression Using Ferrous Sulfate

Camden Drake Shelton

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David S. McCall. Chair

Shawn D. Askew. Co Chair

Erik H. Ervin. Member

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ABSTRACT

Dollar spot is one of the most common diseases of warm- and cool-season turfgrass stands and is especially devastating on creeping bentgrass (*Agrostis stolonifera* L.). The fungus *Sclerotinia homoeocarpa* degrades the foliage by creating silver, dollar-sized depressions of dead and bleached turf. Frequent fungicide applications and cultural management strategies are required throughout the growing season to prevent or reduce severity of this disease. Previous research has demonstrated that ferrous sulfate applied at 48.8 kg ha^{-1} suppresses dollar spot epidemics without traditional fungicides. *In vitro* studies showed 100 to 1,000 mg kg^{-1} of ferrous sulfate directly suppressed *S. homoeocarpa* growth of an isolate collected from an established, intensively-maintained creeping bentgrass putting green. Genetic diversity of *S. homoeocarpa* segregates isolates into two groups; strains generally associated with warm-season and cool-season grasses. It is unknown whether isolates of each group react similarly in the presence of ferrous sulfate. Our research explored use rates of ferrous sulfate required to suppress 50% of dollar spot in the field and *in vitro*. Ferrous sulfate (heptahydrate 20% Fe, Valudor Products Inc) rates in field trials included 0, 4.88, 24.4, 48.8, and 97.6 kg ha^{-1}. Our results indicate a hyperbolic relationship between ferrous sulfate rate and dollar spot reduction. Using this model, 26.4 kg ha^{-1} reduced dollar spot incidence by 50%. We concluded that ferrous sulfate suppresses 50% of *S. homoeocarpa* mycelial growth at between 480 and 720 mg L^{-1} concentration in 0.25 strength potato dextrose agar *in vitro*, and fungitoxic activity of ferrous sulfate was dependent primarily on historical fungicide inputs at isolate collection sites. The use of ferrous sulfate may supplement traditional fungicide use. Chlorothalonil is the most common fungicide used to suppress dollar spot in turfgrass. Annual site-use limitations of chlorothalonil often prevent turf managers from achieving acceptable dollar spot control throughout the season. It is not known how ferrous sulfate may contribute to a successful chlorothalonil fungicide program. Therefore, we examined whether ferrous sulfate can be used to minimize chlorothalonil requirements through reducing active ingredient concentrations and extending the longevity, while still maintaining acceptable disease control. Chlorothalonil treatments were applied at 0, 2.28, 4.57, 6.86, and 9.16 kg ai ha^{-1} (Daconil WeatherStik) across plots treated with and without 48.8 kg ha^{-1} ferrous sulfate applied bi-weekly. Ferrous sulfate reduced the chlorothalonil rates necessary for 80% disease reduction by 36 to 51% across all trials. Additional studies showed that ferrous sulfate applied with chlorothalonil increased duration of disease control by five days and eliminated two seasonal treatments. Our research expands the guidelines for practical ferrous sulfate usage for dollar spot suppression by elucidating the rate-to-disease relationship and providing best management practices involving admixtures with chlorothalonil.

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GENERAL AUDIENCE ABSTRACT

Turfgrass systems offer many environmental and recreational benefits. Managing turfgrass stands that are free of damaging turf pests is essential to providing aesthetically pleasing lawns, golf courses, and sports fields. Creeping bentgrass is one of the most common turfgrass types found on golf course putting greens but is also used on golf course fairways and tee boxes. There are many diseases that can be found on creeping bentgrass when environmental conditions are favorable. Of these diseases, dollar spot is the most common. When dollar spot is present, half-dollar sized spots of bleached turf can be seen. In order to prevent these easily noticeable spots from appearing, fungicide applications are required in a given growing season to prevent the pathogen from infecting. Available fungicides are very effective at providing control but can be very costly. Beyond fungicide use, other research has shown various cultural practices to reduce disease incidence. Previous research has shown that iron sulfate applied to creeping bentgrass can reduce dollar spot epidemics without the use of fungicides. Laboratory studies have shown a similar trend as ferrous sulfate at varying concentrations directly suppressed dollar spot pathogen growth. In both cases, a limited range of ferrous sulfate rates was tested. To obtain more information we explored use rates of ferrous sulfate required to suppress 50% of dollar spot in the field and *in vitro*. Ferrous sulfate rates in field trials ranged from 0 to 97.6 kg ha^{-1}. Results from these trials were used to create a hyperbolic regression. Using this model, we were then able to determine that 26.4 kg ha^{-1} iron sulfate was required to suppress 50% of the dollar spot in the field. For the laboratory studies we concluded that ferrous sulfate suppresses 50% of the dollar spot pathogen mycelial growth between 480 and 720 mg L^{-1} iron sulfate concentrated potato dextrose agar. Although there are many different fungicides available for dollar spot control, the active ingredient chlorothalonil is the most common used. Due to the mode of action which chlorothalonil exhibits, it is much less likely that the pathogen causing dollar spot can become resistant. Although resistance is not an issue, governmental annual site-use limitations restrict turf managers from achieving desirable control. The use of iron sulfate in conjunction with chlorothalonil has not been previously studied. Chlorothalonil treatments were applied at a range of labeled use rates across plots treated with and without 48.8 kg ha^{-1} ferrous sulfate applied bi-weekly. Ferrous sulfate reduced the chlorothalonil rates necessary for disease reduction. If a threshold of 80% is used, up to 50% reduction in chlorothalonil use was observed. Supplemental studies investigating the duration of control achieved by the combination showed an increase of up to 5 days and eliminated the need for two applications across one season. This research fills a huge gap in our knowledge base on the practical use of iron sulfate for dollar spot control.

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Chapter 1: Literature Review

Introduction

Dollar spot is a disease of turf caused by the fungal pathogen *Sclerotinia homoeocarpa* F.T. Bennett. It is one of the most prevalent and consistent diseases of both warm- and cool-season turfgrasses around the world (Smiley et al., 2005). While this pathogen can infect most grasses grown for turf applications, it is especially devastating to closely mown putting green surfaces and fairways. Creeping bentgrass (*Agrostis stolonifera* L.) and annual bluegrass (*Poa annua* L.) are the prominent species used for closely mown turf in the northern United States and Canada (Goodman and Burpee, 1991). The proper use of fungicides in conjunction with sound cultural practices allows turf managers to maintain putting greens and fairways free of dollar spot within consumer expectations (Vincelli et al., 2014). Regardless of fungicide effectiveness, clientele and societal expectations encourage them to adopt IPM approaches over the use of synthetic fungicides (Fidanza et al., 1996).

Cosmetic pesticide use for turfgrass has been publicly scrutinized since the 1960's (Couch, 1995). Scrutiny has increased as environmental and health groups advocate for increased regulation of pesticides used in lawn-care (Pralle, 2006). Cosmetic pesticides have been banned in metropolitan areas, such as Ontario, Canada (Cole et al., 2011) and more recently in Montgomery County, Maryland (Hamlin, 2014). Researchers have responded by exploring alternative dollar spot suppression strategies to supplement synthetic chemical controls.

Overview of *Sclerotinia homoeocarpa*

Dollar spot was first described by Monteith and Dahl, 1932 and considered to be a *Rhizoctonia* sp*.* In 1937 F.T. Bennett observed different isolates of the pathogen from varying regions and determined that the fungus was a new species of *Sclerotinia*, which was later assigned as *S. homoeocarpa* (Bennett, 1937). Symptoms of this disease on closely mown golf course putting greens and fairways include small, circular, sunken patches of bleached turf about the size of a silver dollar. Due to these characteristics, the common name "dollar spot" was given (Bennett, 1937). While dollar spot was originally classified as *S. homoeocarpa* in 1937, many plant pathologists argued that the pathogen does not fit in this classification. Research by Beirn et al (2013) confirmed that dollar spot does not belong to the Sclerotiniaceae family. This research along with Aynardi et al, (2016) have determined there are two distinct species capable of causing the disease. Type I and Type II isolates are capable of producing disease on both warm and cool season grasses with Type 1 isolates being the most virulent (Aynardi et al., 2016). Further research is needed to taxonomically identify the appropriate species for the fungus (Vargas, 2005). For the remainder of this review however, the causal agent of dollar spot will be referred to as *S. homoeocarpa. S. homoeocarpa* is an ascomycete fungus which does not form sclerotia but instead produces pigmented stromata. No spores are produced by the pathogen allowing spread to occur only by mycelial growth from individual infection centers. *In vitro*, stroma (black colored) will form in 2-4 weeks after plating into potato dextrose agar. Fast-growing, fluffy-white mycelium will also follow shortly after plating and may turn colors (Smiley et al., 2005).

In addition to the symptoms listed above, mycelial growth of *S. homoeocarpa* can be observed in the field. This is especially prominent when dew is present on the leaves in the early morning. As dew evaporates, the mycelium dry and are no longer seen. Leaf blades infected by the pathogen develop reddish brown to tan colored lesions mimicking the shape of an hourglass. Without proper treatment, the infected turf will senesce. The patches caused by infection are normally 2.5 cm or smaller in size on closely mown turf. These patches may also coalesce causing larger and more irregularly shaped patches (Smiley et al., 2005). When this occurs, recovery of infected areas may take weeks (Monteith and Dahl, 1932; Smiley et al., 2005).

Dollar spot is capable of developing on most maintained turfgrass species, including *Agrostis*, *Festuca*, *Lolium*, *Poa*, *Cynodon*, and *Zoysia* species (Smiley et al., 2005). Due to its smooth uniform surface, creeping bentgrass is the most widely used turfgrass on putting greens (McCarty et al., 2007). The pathogen maintains virulence across the entire environmental range of creeping bentgrass (Smiley et al., 2005). Environmental conditions are most favorable for *S. homoeocarpa* fungal development during warm, humid days followed by cool nights. Air temperatures ranging from 15-30°C coupled with the humidity and heavy dew associated with spring, summer, and fall in the transition zone favors mycelial growth (Smiley et al., 2005). Optimal growth of S*. homoeocarpa* occurs at 21 to 27°C and an atmospheric humidity of 85% and above (Walsh et al., 1999).

Improper irrigation practices timed from mid-morning through the evening will extend periods of leaf wetness therefore increasing the chance for infection (Smiley et al., 2005). Cool nighttime temperatures associated with early and late summer result in heavy morning dew which increases the chance for dollar spot occurrence (Walsh et al., 1999). During prolonged favorable conditions, mycelia comes into contact with a moist leaf surface, penetrates the leaf surface, and infects the leaf blade. The fungus may also enter the plant through wounds created by mowing which occurs frequently on putting greens and fairways. (Smiley et al., 2005).

When the environment favors disease development, *S. homoeocarpa* can infect turf species and spread (Smiley et al., 2005). Following penetration through the cuticle, the pathogen releases oxalic acid that causes necrosis of the apical meristem on varying turfgrass types (Venu et al., 2009). Oxalic acid produced by the pathogen is required for pathogenicity and causes programmed plant cell death. Research has shown the amount of oxalic acid present depends on the pH of the pathogen's growing environment (Venu et al., 2009). Excreted oxalic acid combines with calcium in the cell wall to aid fungal intrusion (Venu et al., 2009; Riou et al., 1991).

Creeping Bentgrass Susceptibility to Dollar Spot

Creeping bentgrass is a perennial grass species that is commonly used on golf greens, tees, and fairways. Creeping bentgrass also persists as a weed in other turfgrass stands of different species (Vargas and Turgeon, 2004). It spreads by stolons and is not considered a prolific seed producer. When grown as a turfgrass in the transition zone, it is very prone to infection by *S. homoeocarpa* (Christians, 2007). Due to this, creeping bentgrass has been bred for reduced dollar spot sensitivity. Dollar spot susceptibility varies considerably among commercially available cultivars (Lee et al., 2003). Reduced sensitivity of creeping bentgrass to *S. homoeocarpa* infection may be attributed to either stomatal reduction or by increasing trichome size (Bonos et al., 2004). Cultivars with more stomates per area are less resistant to pathogen infection than those with fewer stomates. The stomates allow *S. homoeocarpa* to gain entry into the plant (Monteith and Dahl, 1932). Research by Bigelow et al. (2011) has shown cultivars of ‘007’ and ‘Tyee’ to be the most resistant to infection and cultivars such as ’96-2’ and ‘A-4’ to be the most susceptible

Creeping bentgrass varieties that are less susceptible to dollar spot still require additional inputs when conditions are favorable for disease development. Highly susceptible bentgrass cultivars may have desirable traits, but typically require increased fungicide applications, even during periods of low disease pressure. Highly resistant cultivars allow for a more flexible fungicide program, even during periods of high disease pressure (Settle et al., 2001).

Pesticide Use Limitations in Turf Management

Federal, state, and local government regulations and increased environmental concerns over pesticides have led to increased focus on alternative dollar spot management strategies. The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) gives the Environmental Protection Agency (EPA) the authority to regulate pesticide usage in the United States. Since the early 2000’s, regulations on pesticides in North America have been adopted by many precincts (Be´lair et al., 2010).

Canada was one of the first to adopt a government mandated pesticide ban. Municipalities such as Que´bec established laws that prevented any pesticides from being used on residential lawns as early as 2003 (Be´lair et al., 2010). The City of Ontario Canada established regulations in 2009 that had a huge impact on the turf industry there (Anonymous ,2009). This required golf courses to comply with strict regulations in order to continue using pesticides.

In general, more pesticides are available for use in turfgrass in the United States compared to other countries (Be´lair et al., 2010). However, New York and California have imposed strict pesticide registration regulations. The insecticide, imidacloprid, cannot be used on Long Island in New York. Additionally, a statewide ban in New York prohibits the use of synthetic pesticides on school properties (Grant, 2011).

Although there are effective chemical controls for managing most turfgrass diseases, there is much research testing alternative control options. Other than changes in legislation, there are other reasons to search for alternatives. *S. homoeocarpa* has shown cross and multiple resistance to more than one fungicide (Vargas, 1994). Research has shown that *S. homoeocarpa* populations on turfgrass can shift rapidly and become resistant to systemic fungicides (Young-Ki et al., 2008). Beyond fungicide resistance and legislation, a search for alternative controls which are more economical are welcomed by turf managers.

Dollar Spot Control and Alternatives

Chemical Control

Fungicide inputs are a requisite to maintain a creeping bentgrass putting green or fairway that meets the high expectations of golfers (Jo et al., 2008). Multiple fungicide applications are required throughout the growing season to suppress *S. homoeocarpa* populations to acceptable levels. While there are numerous fungicides on the market that offer dollar spot control, fungicide resistance and governmental regulations have reduced the chemical control options available. According to Latin et al (2012), the fungicide classes that are most effective at controlling dollar spot are: aromatic hydrocarbons, benzimidazoles, carboxamides, nitriles, QoIs, dicarboximides, DMIs, and phenylpyrolles.

Many of these fungicides that controlled dollar spot when they were released are now resistant to one or more chemical classes (Jo et al., 2008). This is partly due to the persistent nature of this disease which requires repeat applications throughout the growing season. *S. homoeocarpa* is also a prolific mycelial producer, which increases the potential for resistant populations (Latin, 2012). Five chemical classes that turf managers have relied on for years will no longer suppress dollar spot because of resistant populations. To help correct this dilemma, companies have brought more succinate dehydrogenase inhibitors (SDHI) to market. This group of fungicides was thought to be less likely for *S. homoeocarpa* resistance (Latin, 2012). However, new research has demonstrated that certain isolates of S. homoeocarpa have reduced sensitivity to certain SDHI chemistries after repeated field use (Popko et. al, 2016).

An increased reliance on multi-site mode-of-action fungicides such as chlorothalonil and fluazinam is likely to occur as dollar spot resistance to single-site fungicides occur more frequently. Multi-site fungicides require more than one mutation for that mode-of-action to become ineffective for dollar spot control. Chlorothalonil, a nitriles fungicide class, can provide acceptable levels of dollar spot control in creeping bentgrass turf, but requires frequent and high application rates for consistent control. The rate and application intervals for this fungicide are restricted, which can limit its use as a main fungicide for dollar spot control (McDonald et al., 2006).

Cultural Control

Plant health plays a huge role in a pathogen's ability to attack a plant. (Smiley et al., 2005). Nitrogen is an essential macronutrient used for plant growth and development. A lack of nitrogen in leaf tissue will predispose the plant foliage to disease infection. Vigorous plant growth will reduce the disease severity caused by *S. homoeocarpa* (Markland et al., 1969). A more productive turf will require a more frequent mowing regime which may remove necrotic tissue (Walsh et al., 1999).

The amount and duration of dew presence on plant foliage plays a role in dollar spot development (Williams et al., 1996). Practices such as mowing and rolling implemented by turf managers which can minimize the leaf wetness period and in turn minimize dollar spot disease occurrence (Ellram et. al, 2007). Creeping bentgrass grown on soils with inadequate moisture can also increase the chances for dollar spot development (Couch, 1960).

Although cultural practices can aid in dollar spot control, generally they are not effective alone. An integrated pest management approach which combines cultural and chemical control practices is the most effective. Combining these control strategies can increase fungicide application longevity and control. Previous research has investigated fertilizer source for dollar spot control (Markland et al., 1969), but little research has investigated the effects of ferrous sulfate on control of dollar spot or other turf pathogens (Reams et al., 2013).

Ferrous Sulfate

The use of elemental sulfur and iron for disease control dates back to the early 1800's. Sulfur alone was the first fungicide applied to control powdery mildew (Maloy, 1993). The use of sulfur for dollar spot however was shown to be ineffective (Monteith and Dahl, 1932). The use of iron for dollar spot control was first observed when applying sewage sludge to creeping bentgrass. It was noted that the iron, copper and zinc levels were increased in plant tissue and as a result, dollar spot incidence was decreased (Markland et al., 1969). Another study observing the effect of ferrous sulfate on turfgrass nutrition observed disease and weed reduction in treated plots (Morgan, 1982). In this study, no negative impact to turf plant health was observed through three years of repeat applications.

In production agriculture, a study by Forsyth et al, (1957) showed the combination of iron and sulfur (ferrous sulfate) to reduce the amount of rust in wheat (*Puccinia graminis)*. In this study, the iron ions were found at the site of infection which led the authors to conclude that iron plays a role in defense against potential pathogens. Although science has proven iron to be effective against some pathogens, research has also show iron to have a reverse effect against other pathogens. Fusarium requires iron to create an enzyme which enables the pathogen to invade the plant (Graham, 1983).

Repeated, high application rates of ferrous sulfate (48.8 kg ha^{-1}) have been observed to control or reduce dollar spot infection on creeping bentgrass and microdochium patch on annual bluegrass putting greens (McCall et. al, 2016 and Mattox et. al, 2016). Beyond these studies, no literature is available on the effects of iron on turf fungal pathogens.

Research Objectives

It is unknown how different rates of ferrous sulfate impact dollar spot development beyond rates reported by McCall et.al (2016). Our research further investigates the ability of ferrous sulfate to suppress dollar spot both *in-situ* and *in-vitro*. Minimizing chlorothalonil usage without compromising dollar spot efficacy will become increasingly important amid public scrutiny and potential additional federal restrictions. For these reasons, our research investigates how ferrous sulfate can impact chlorothalonil use rates and frequency needed to suppress dollar spot.

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Chapter 2

Title: Reducing Chlorothalonil Inputs for Dollar Spot Suppression Using Ferrous Sulfate

Authors: Camden Shelton, Virginia Tech (cam1@vt.edu) David McCall Ph.D., Erik Ervin Ph.D., Shawn Askew Ph.D.

Keywords: dollar spot, ferrous sulfate, chlorothalonil

Abstract:

Chlorothalonil, a multi-site contact fungicide, is commonly used to suppress the dollar spot pathogen both preventatively and when disease levels become unacceptable. Annual site-use limitations of chlorothalonil can prevent turf managers from achieving acceptable dollar spot control throughout the season when relied upon solely. Previous research has demonstrated that ferrous sulfate applied at 48.8 kg ha^{-1} can suppress dollar spot epidemics without traditional fungicides, but how this may impact a chlorothalonil fungicide program is not known. This research explores whether adding ferrous sulfate to a chlorothalonil fungicide program can maximize control achieved. Trials were established focusing on rate response of the combination and longevity. Chlorothalonil treatments were applied as follows: 0, 2.28, 4.57, 6.86, and 9.16 kg ai ha^{-1} (Daconil WeatherStik) across plots treated with and without 48.8 kg ha^{-1} ferrous sulfate (heptahydrate 20% Fe, Valudor Products Inc) applied bi-weekly. Chlorothalonil rates required to suppress disease populations by 80% ranged from 2.8 to 5.6 kg ai ha^{-1} in the presence of ferrous sulfate and 6.0 to 8.9 kg ai ha^{-1} when plots were not sprayed with ferrous sulfate. Additional research indicates that adding routine ferrous sulfate applications to a chlorothalonil fungicide program may extended the longevity of control. Additional research indicates that adding routine ferrous sulfate applications to a chlorothalonil fungicide program may extended the longevity of control. The re-application interval of chlorothalonil when applied without ferrous sulfate was significantly longer compared to plots treated with ferrous sulfate. The ferrous sulfate program utilized in these studies reduced chlorothalonil inputs by two applications across a single season. This research provides turf managers with more practical applications of ferrous sulfate for disease control.

Introduction

Dollar spot is a destructive disease of creeping bentgrass (Agrostis stolonifera L.) putting greens and fairways caused by the fungal pathogen *Sclerotinia homoeocarpa* (F. T. Bennett). Creeping bentgrass is the most widely used turfgrass species on golf courses (Jo et al., 2008). Turfgrass mangers utilize considerable resources to prevent dollar spot from exceeding unacceptable levels of injury. Symptoms of dollar spot are easily noticeable and unsightly. *S. homoeocarpa* creates sunken and circular patches of brown colored tissue which are generally equal to the size of a silver dollar (Couch, 1995). Fungicides are commonly applied to prevent dollar spot development (Vargas, 1994).

Active ingredients that control dollar spot from seven biochemical modes of action are available to turfgrass managers in the Unites States (Couch, 1995). Five of these modes of action are active at a single biochemical site of fungal growth. While single-site fungicides typically suppress dollar spot for longer, they are often more prone to fungal populations becoming resistant by genetic mutations to avoid site-specific inhibition (Vargas, 1994). Fungal populations are less likely to overcome inhibition from multi-site fungicides (Latin, 2015). Extensive use of multi-site fungicides are used out of necessity on sites where fungal populations have evolved resistance to single-site modes of action. Chlorothalonil, a multi-site mode of action fungicide, is the most widely used fungicide in the turf industry (Vargas, 2004). This fungicide is effective at suppressing dollar spot with a low probability of populations developing resistance. Chlorothalonil has been used extensively on turfgrasses since the 1960's with no reported cases of resistance (Burpee, 1997; Vincelli and Munshaw, 2014).

In 1999 however, the U.S. Environmental Protection Agency placed use restrictions on chlorothalonil in turfgrass sites thus reducing its overall effectiveness for disease suppression (Vincelli and Dixon, 2003). These regulations restricted chlorothalonil use from home lawns and reduced individual application and season total chlorothalonil amounts allowed on golf courses and sod farms. Since these restrictions were imposed, some research began evaluating methods to improve chlorothalonil performance (McDonald et al., 2006). Improving chemical performance and discovering alternatives to chemical control will be a key part to overcoming fungicide resistance and improving dollar spot management strategies (Jo et al., 2008).

Cultural management practices are often capable of reducing dollar spot but are not relied solely upon because they are not completely effective (Bonos et al., 2004). Cultural strategies with documented dollar spot reductions include dew and free water management, sufficient nitrogen fertilization, and thatch management (Williams, 1996 and Couch, 1995; Markland et al., 1969).

The use of ferrous sulfate has shown the ability to suppress dollar spot but is generally insufficient alone (McCall et al., 2017). This research determined that ferrous sulfate has a direct fungitoxic effect on *S. homoeocarpa.* Other research has shown reductions in *Microdochium nivale* following applications of ferrous sulfate (Mattox et al., 2016). Other research dating back to the 1980's observed disease reductions following ferrous sulfate applications (Graham, 1983).

To the best of our knowledge, no previous research has investigated chlorothalonil and ferrous sulfate in combination for dollar spot control. Previous research has only evaluated ferrous sulfate at 48.8 kg ha for dollar spot suppression. How ferrous sulfate may impact dollar spot at higher or lower rates is not well understood. Therefore the objectives of this research were to examine the influence of ferrous sulfate on chlorothalonil rates required to suppress dollar spot and to determine whether ferrous sulfate can increase the longevity of chlorothalonil applications targeting dollar spot and, thereby, minimize applications needed throughout the season.

Materials and Methods:

Chlorothalonil rate field study. Four field trials were conducted in Blacksburg, VA between June and August of 2016 and 2017 on three creeping bentgrass stands. Trials for both years were conducted on a mature '007' creeping bentgrass putting green at the Virginia Tech Turfgrass Research Center, a one-year-old '96-2' creeping bentgrass fairway in 2016 and established 'L-93' creeping bentgrass fairway in 2017 at the Glade Road Research Facility. The locations with mature bentgrass stands had a history of heavy dollar spot infestation. Dollar spot incidence was high in '96-2' plots compared with other cultivars in variety evaluation study (Bigelow et al., 2011). The '96-2' fairway suffered severe late-summer drought damage in 2016 which required re-establishment of the site. The second year of trials on fairways was moved to ‘L-93’ because the original site was not fully established at trial initiation. The research putting green was built to USGA specifications (90% sand, 10% peat moss) and mowed five times a week at 3.2 mm with clippings removed. The putting was core aerified twice per year in the fall and spring to remove 15% of surface area and back-filled with sand immediately after core removal. Granular slow-release nitrogen sources were used immediately after aerification in the spring and fall totaling 97 kg N ha^{-1} yr^{-1}. Supplemental nitrogen (46-0-0) was applied biweekly throughout the study at a rate of 7.3 kg N ha^{-1} totaling 43.8 kg N ha^{-1} yr^{-1}. Fairways were established on silt loam and mowed three times per week at 16.5 mm. All trial sites were irrigated as needed to prevent visual signs of wilt.

Five rates of chlorothalonil (Daconil WeatherStik, Sygenta Professional Products, 72.4 kg ai ha^{-1}) and two rates of ferrous sulfate (Ferrous sulfate heptahydrate 20%Fe, Valudor Products Inc.) were arranged in a 2 x 5 factorial design and completely randomized within blocks over four replications to evaluate efficacy against dollar spot. All treatments were applied bi-weekly as liquid suspension in water with a CO_2-pressurized (275.6 kPa at 814 L ha^{-1} and 407 L ha^{-1}) backpack sprayer through TeeJet TTI 11004VP nozzles. Treatments included chlorothalonil rates of 0 kg ai ha^{-1}, 2.28 kg ai ha^{-1}, 4.57 kg ai ha^{-1}, 6.86 kg ai ha^{-1}, and 9.16 kg ai ha^{-1} and rates of 0 kg ha^{-1} and 48.8 ha^{-1} ferrous sulfate heptahydrate. Ferrous sulfate and chlorothalonil were independently because of tank mixture incompatibility. Ferrous sulfate treatmetns were sprayed immediately following chlorothalonil applications. Plot size measured 1.8m x 1.8m.

Dollar spot infection center counts, visual estimation of percent disease, and visual turf quality were collected weekly for 12 weeks following trial initiation. Turf quality were based on a 1-9 visual estimation scale with 1 = dead, poor quality turf, 6 = minimally-acceptable turf, and 9 = healthy, high quality turf (Morris, 2002). Infection centers were counted with the aid of a 0.9m x 1.8m sampling grid with 5cm spacing. Percent dollar spot infestation was estimated using methods described by Tredway et. al (2001).

Dollar spot counts and percent disease were transformed using the area under disease progress curve (AUDPC) to quantify dollar spot intensity over the duration of the study (Madden et. al, 2007):

$$AUDPC = \left\{ \sum_{i=1}^{n-1} \left[\left(y_i + y_{i+1} \right) / 2 \right] * \left(t_{i+1} - t_i \right) \right\}$$

Turf quality was transformed using a standardized AUTQPC to represent daily estimates throughout the duration of the study. Data were subjected to analysis of variance (ANOVA) using the general linear models procedure in SAS, version 9.3 (SAS Institute, Cary, NC) and included chlorothalonil rate, ferrous sulfate rate, year, location, and their interactions. Regression analysis was determined using goodness-of-fit for each data set using transformed AUDPC from 12 weekly ratings. Both linear and hyperbolic regression models were created based on goodness-of-fit. The best fit was determined with the least sum of squares using PROC NLIN (SAS v9.3) using the Gauss-Newton method with estimated parameters for each trial listed in Table 2. Visual estimates of percent dollar spot and turf quality were compared as dependent variables against infection center counts using general linear regression.

Chlorothalonil longevity. Field trials were established to determine whether a ferrous sulfate program can extent the longevity of chlorothalonil control of dollar spot on previously described ‘L-93’ fairway and ‘007’ putting green creeping bentgrass stands. Ferrous sulfate (Ferrous sulfate heptahydrate 20% Fe, Valudor Products Inc.) was applied bi-weekly at 48.8 kg ha^{-1} and compared against a non-treated control across four replications in a randomized complete block design. Chlorothalonil was applied to plots at 8.20 kg ai ha^{-1} when the number of infection centers became greater than 30 per plot. Chlorothalonil was applied immediately after data were collected and before ferrous sulfate, if applicable. All treatments were applied bi-weekly with a CO_2-pressurized (275.6 kPa at 814 L ha^{-1}) backpack sprayer through TeeJet TTI 11004VP nozzles. Individual plots measured 1.8m x 1.8m.

Data for dollar spot infection centers were counted twice weekly for 12 weeks, as previously described. Chlorothalonil was applied only on days when mean dollar spot counts exceeded a threshold of 30 infection centers per plot within main effect of ferrous sulfate treatment. Dollar spot infection centers were transformed using AUDPC as previously described to determine overall main effect differences. Mean interval (days) between all applications of chlorothalonil was calculated for each treatment within replication. Data were subjected to analysis of variance (ANOVA) and means were separated, when appropriate, using the general linear models procedure in SAS, version 9.3 (SAS Institute, Cary, NC).

Results: Chlorothalonil rate field study

Dollar spot pressure varied considerably across trials, though the general trend in disease progression was consistent (data not shown). To account for this, AUDPC of each plot was converted to percentage of the untreated control within replication for each trial. The ANOVA test for dollar spot infection centers were highly significant ($P < 0.0068$) for ferrous sulfate, chlorothalonil rate, trial, and their interactions (Table 1). Therefore, data for the interaction between ferrous sulfate and chlorothalonil rate will be presented separately by trial. The greatest variance in our data is attributed to ferrous sulfate ($F = 2019$), with trial also contributing strongly to overall variance ($F = 788$). This F value explains some of the error associated with treatments.

The influence of ferrous sulfate on dollar spot suppression with chlorothalonil is presented by trial (Figure 1). The relationship between dollar spot infection center counts chlorothalonil rates was linear for each trial in the presence of a baseline ferrous sulfate program ($r^2 \geq 0.96$). A hyperbolic relationship exists between dollar spot reduction and chlorothalonil rate without ferrous sulfate in trials

1, 2, and 4 (*pseudo-r^2* ≥ 0.96). Linear regression was a better fit between dollar spot reduction and chlorothalonil rate without ferrous sulfate in trial 3 (r^2 = 0.79). These data were used to generate the effective chlorothalonil concentration required to suppress dollar spot by 80% with and without an accompanying ferrous sulfate program. Means generated for chlorothalonil rate required to suppress populations by 80% alone and in the presence of ferrous sulfate are shown in Table 2. Effective chlorothalonil concentrations needed to suppress 80% of dollar spot incidence were significantly lower when applied in conjunction with a ferrous sulfate program than when applied alone. All generated means comparing iron to no iron were significantly different. Dollar spot was suppressed by greater than 80% with ferrous sulfate alone on the '96-2' fairway site (Figure 1). Our model for '007' putting green in 2016 estimates an 8.7 kg ai ha^{-1} and 4.1 kg ai ha^{-1} rate of chlorothalonil required to suppress dollar spot populations by 80% for no ferrous sulfate and with ferrous sulfate, respectively. Estimations were similar on the '007' putting green in 2017, with chlorothalonil rates of 10 without supplemental ferrous sulfate and 5.7 kg ai ha^{-1} ferrous sulfate treated plots. Our 'L-93' fairway site had similar results to putting greens trials with estimated chlorothalonil rates of 7.8 and 4.3 kg ai ha^{-1} needed to suppress dollar spot by 80% without ferrous sulfate and with ferrous sulfate, respectively. Rates of chlorothalonil necessary for an 80% reduction in dollar spot were 45-55% lower when used in combination with a ferrous sulfate program for three of four trials, with ferrous sulfate alone being sufficient in the fourth trial.

Discussion: Chlorothalonil rate field study

This research demonstrates that routine applications of ferrous sulfate (48.8 kg ha^{-1}) can lower chlorothalonil use rates required to suppress dollar spot populations. However, the extent of additional suppression is dependent upon overall disease pressure and what is considered acceptable for a given site during an epidemic. Disease pressure was high in three of our four trials, but very low in the fourth.

Ferrous sulfate alone reduced dollar spot pressure by greater than 55% in all trials and by greater than 80% at our site with low pressure. We used an infection center reduction of 80% in our research to provide practical guidelines on how best to incorporate ferrous sulfate into a chlorothalonil program. Many studies have shown that chlorothalonil alone will not provide complete control of dollar spot for 14 days under heavy disease pressure (cite PDMR reports or something…see next sentence comment). For instance, Fidanza et al, (2016) reported a 36% reduction with chlorothalonil throughout the season when applied as late preventative and repeated throughout the season. Popko and Jung (2014) reported only a 30% reduction in dollar spot infection centers with chlorothalonil when applied curatively. Dollar spot was active throughout our research in chlorothalonil-treated plots and applications were therefore considered curative for much of the season. Other studies testing chlorothalonil for dollar spot control have shown chlorothalonil to suppress disease incidence to much lower levels (McDonald et al., 2006). McDonald et al. indicated an unacceptable threshold of 1% disease within plots. Dollar spot pressure was higher in three of our studies than in those of McDonald et al. (2006). In our research, a 1% threshold would correspond with 10 infection centers per plot for these three trials (data not shown). This threshold would be unrealistic in our study because 11.5-15 kg ai ha^{-1} would be needed to suppress dollar spot. This is well above legal chlorothalonil limits and would never be a logical option for turf managers.

Generally, ’96-2’ creeping bentgrass is more susceptible to dollar spot than ‘L-93’ (Bigelow et al., 2011). Dollar spot pressure was unexpectedly low on the ’96-2’ fairway site in 2016, based on historical cultivar susceptibility (Bigelow et al., 2011). Our test site was a newly established creeping bentgrass fairway which received higher nitrogen inputs than the ‘L-93’ creeping bentgrass fairway evaluated in 2017 which may explain why less dollar spot was observed. The ’96-2’ fairway was seeded in the spring of 2016 and required additional nitrogen inputs to reach maturity in time for our field studies. Ferrous sulfate was effective at reducing dollar spot by 80% alone at this test site. This data supports the elimination of chlorothalonil on fairways with low risk of dollar spot development. Ferrous sulfate may be sufficient to suppress dollar spot with cultivars with historically low dollar spot susceptibility, such as ‘Declaration’ and ‘Memorial’ (Bigelow et al., 2011).

The use of ferrous sulfate in combination with chlorothalonil provides an acceptable alternative to achieve acceptable dollar spot suppression. Chlorothalonil use limitations differ on putting greens and fairways. Data generated from these studies will help turf managers avoid sole reliance on fungicides for dollar spot control. Our data show up to 55% percent reductions in the amount of chlorothalonil required to suppress disease populations to 80%. A 7.75 kg ai ha^{-1} chlorothalonil rate was required to reduce disease levels to 80 percent on our ‘L-93’ fairway site in the absence of ferrous sulfate. Label restrictions allow for 26 kg ai ha^{-1} per growing season on golf course fairways. The maximum allowable three applications of 7.75 kg ai ha-1 chlorothalonil would be insufficient for season-long dollar spot suppression. The addition of a supplemental ferrous sulfate program reduces chlorothalonil rates to 4.3 kg ai ha^{-1} for equal dollar spot suppression. Reducing the chlorothalonil rate allows for an additional three applications while still staying within label restrictions.

In instances where more than 80% disease control is desired, a higher rate of chlorothalonil would be necessary to achieve this. Federal regulations also prohibit more than one application of chlorothalonil above 8.176 kg ai ha^{-1} per year of golf fairways. If 90% disease control was the target desired, a rate of chlorothalonil greater than 8.176 kg ai ha^{-1} would be needed in the absence of ferrous sulfate. This means that 90% or greater control could only be achieved following one chlorothalonil application a year. Our model for the ‘L-93’ fairway predicts that a 90% reduction in disease could be achieved with a multiple application allowable rate of chlorothalonil if incorporated into a ferrous sulfate routine management program.

No injury or phytotoxicity from repeat applications of ferrous sulfate were observed in these trials. One important downfall observed during these studies was the inability to tank-mix chlorothalonil and ferrous sulfate. Dernoden (2001) reported a chlorothalonil flowable incompatible tank-mix with fosetyl-aluminum. When mixing those compounds, a precipitate was formed which caused the chlorothalonil to settle out. Mixing chlorothalonil and ferrous sulfate showed similar results. Regardless of mixing order, the chlorothalonil particles would come out of solution and adhere to the bottom of the spray bottle.

This research shows that adding ferrous sulfate to a chlorothalonil fungicide program can reduce that total amount of active ingredient needed per application for dollar spot control. How ferrous sulfate may impact other fungicide programs is not known.

Results: Chlorothalonil longevity field study

Chlorothalonil was applied six times in plots that did not receive additional ferrous sulfate inputs, whereas, plots receiving routine ferrous sulfate required only four chlorothalonil applications in each replication (Figure 2). These data are not subject to ANOVA because of no variance across replications within treatment. The ferrous sulfate program reduced chlorothalonil inputs by two applications. Chlorothalonil re-application interval was significantly shorter in plots routinely treated with ferrous sulfate ($P = 0.008$). Plots that did not receive ferrous sulfate required chlorothalonil applications every 14.3 days, compared to a 9.9 day interval in conjunction with a supplemental ferrous sulfate program. Dollar spot incidence, as reported by AUDPC, was significantly lower in ferrous sulfate-treated plots than in the chlorothalonil-only treatment ($P < 0.0001$).

Discussion: Chlorothalonil longevity field study

Chlorothalonil can be expected to control dollar spot with a threshold of 1% for about 14 days (Latin, 2006). In these experiments, chlorothalonil controlled dollar spot below 30 infection centers per plot for 9-11 days without the addition of ferrous sulfate. In other research by Shelton et al, 2018 a strong linear relationship was observed showing that infection center counts and percent disease are directly related. Correlating 30 infection centers per plot to percent disease would equal approximately 3% disease per plot for this research. Based on these data, chlorothalonil did not provide equal control duration as compared to Latin, 2006. The difference observed between longevity of fungicide control is likely due to the heavy disease pressure we received in both years of the study.

The data obtained in these experiments tell us that ferrous sulfate is a good addition to a chlorothalonil fungicide program targeting dollar spot. An increase in chlorothalonil duration of control and fewer applications required per season were observed figure 2. Chlorothalonil annual use is restricted to 81.76 kg ai ha^{-1} yr^{-1} on putting greens and 29.12 kg ai ha^{-1} yr^{-1} on fairways. Federal use restrictions also require a 14 day re-treatment interval for rates at and above 8.176 kg ai ha^{-1} on putting greens. For fairway use, a rate greater than 8.176 kg ai ha^{-1} may only be applied once per growing season.

In our research, an 8.176 kg ai ha^{-1} rate would not suppress populations to an acceptable level if applied on a 14-day interval. If ferrous sulfate is applied on a 14-day interval at a 48.8 kg ha^{-1} rate in addition to chlorothalonil at 8.176 kg ai ha^{-1}, dollar spot populations would be suppressed below the set threshold.

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Appendix: Tables and Figures

Table 1: Analysis of variance for the area under disease progress curve (AUDPC) of dollar spot infection centers within creeping bentgrass plots and for ferrous sulfate rate required to reduce dollar spot by 80% (IR_{80}).

	AUDPC		
Source	**DF**	**F Value**	**Pr > F**
rep	3	2.29	0.0817
trial	3	788.45	<.0001
ferrous sulfate	1	2019.22	<.0001
rate	4	193.88	<.0001
ferrous sulfate x rate	4	22.95	<.0001
trial x ferrous sulfate	3	4.26	0.0068
trial x rate	12	20.89	<.0001
trial x ferrous sulfate x rate	12	43.44	<.0001
IR_{80}			
Source	**DF**	**F Value**	**Pr > F**
rep	3	0.5128811	0.2767
trial	3	332.96	<.0001
ferrous sulfate	1	12.49	0.0385
trial x ferrous sulfate	3	57.17	<.0001

Table 2. Chlorothalonil rate needed to suppress 80% of dollar spot infection centers (IR_{80}) alone or in conjunction with a ferrous sulfate program (FS) applied to creeping bentgrass putting greens and fairways in Blacksburg, VA. IR_{80} values calculated using linear and non-linear regression. Asymptote estimated parameter *a* was bounded to be ≤ 100 for biological relevance as higher values are not possible.

	IR_{80} Chlorothalonil kg ai ha^{-1}		**R^2/Pseudo R^2**		**Regression model with estimated parameters**	
Trial	**Alone**	**FS**	**Alone**	**FS**	**Alone**	**FS**
’96-2’ fairway, 2016	4.3	-6.4	0.96	0.79	y = 125x/(1+(125x)/100)	y = 1.44x+88.8
‘007’ green, 2016	8.7	4.1	0.96	0.96	y = 44.7x/(1+(44.7x)/100)	y = 2.87x+68.3
‘L93’ fairway, 2017	7.7	4.3	0.97	0.96	y = 9.73x+5.08	y = 3.66x+64.3
‘007’ green, 2017	10	5.7	0.96	0.98	y = 33.3x/(1+(33.3x)/100)	y = 2.87x+68.3

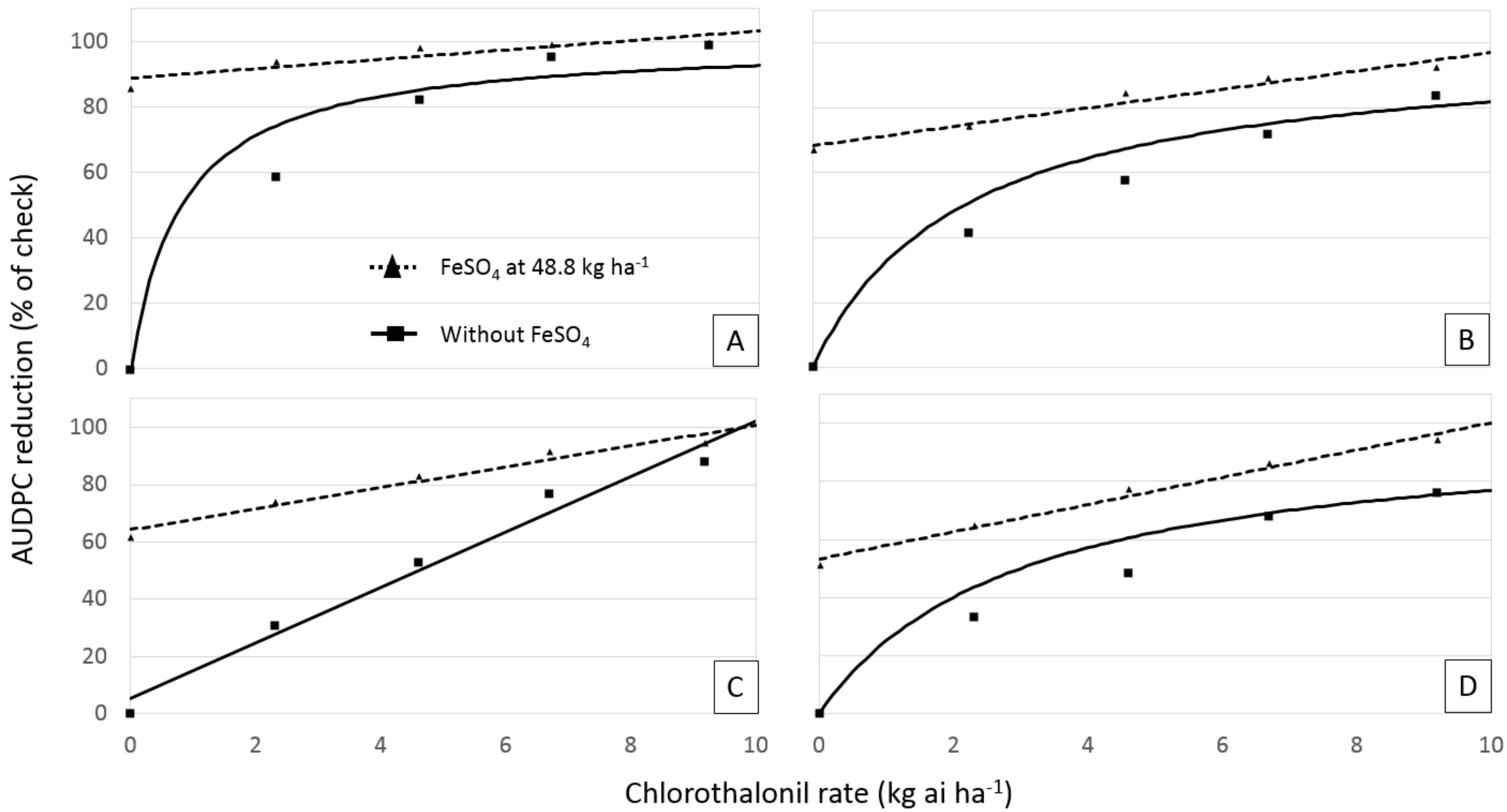

Figure 1: Influence of chlorothalonil rate on area under the disease progress curve (AUDPC) based on 12 weekly infection counts and converted to percentage of untreated plots. Trends are arranged to show significant interactions of iron sulfate 48.8 kg ha^{-1} and four Blacksburg, VA trial sites including A, ’96-2’ creeping bentgrass fairway in 2016; B, ‘007’ creeping bentgrass putting green in 2016; C, ‘L-93’ creeping bentgrass fairway in 2017; and D, ‘007’ creeping bentgrass fairway in 2017. Equations and correlation coefficients and pseudo R^2 estimates for fit of nonlinear equations are shown in Table 1

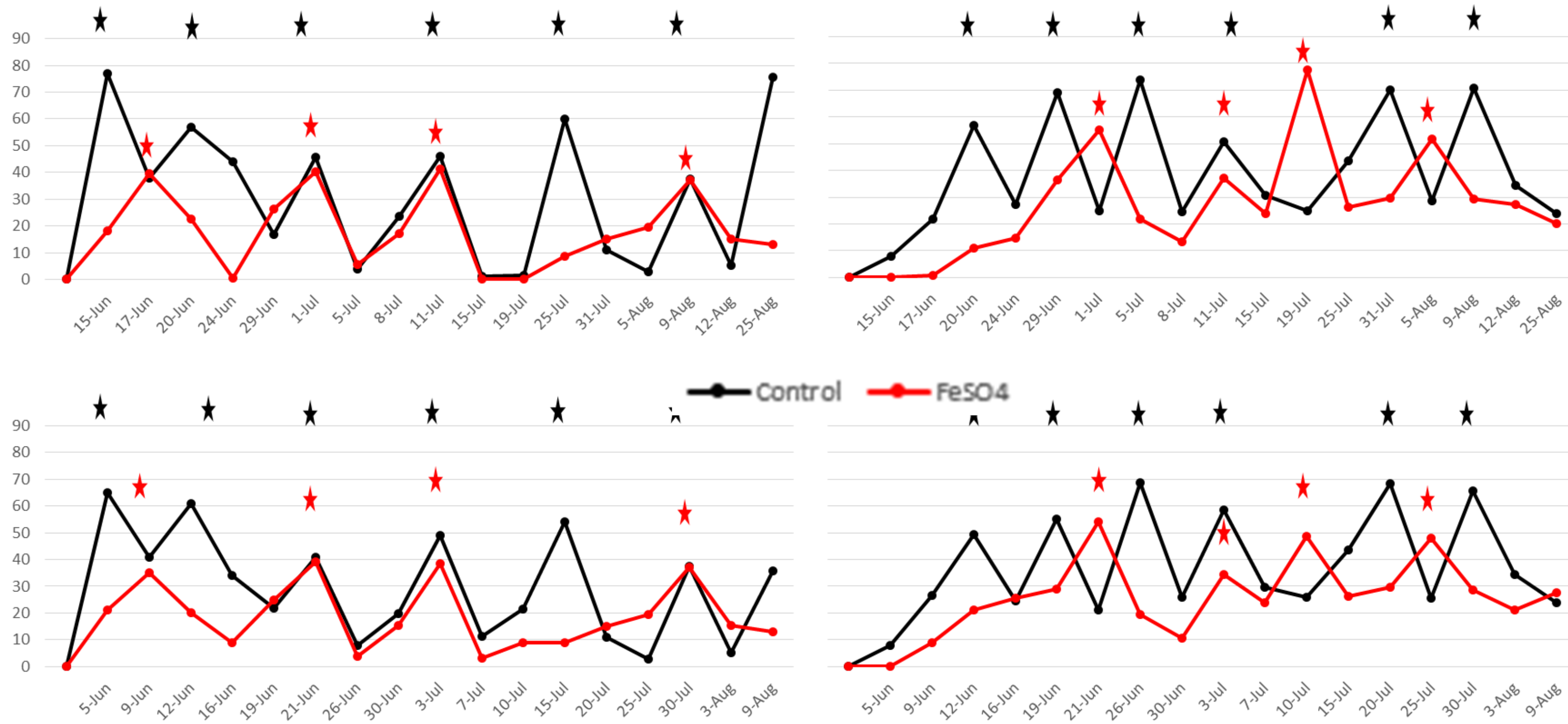

Figure 2: Dollar spot infection center count treatment means across four different trials and two growing seasons. Stars indicate when chlorothalonil was applied to treatments. Red lines represent treatments receiving iron and black lines represent treatments receiving no iron.

Chapter 3

Title: Dollar spot sensitivity to various rates of ferrous sulfate

Authors: Camden Shelton, Virginia Tech (cam1@vt.edu) David McCall Ph.D., Erik Ervin Ph.D., Shawn Askew Ph.D.

Keywords: dollar spot, ferrous sulfate

Abstract:

Dollar spot is one of the most common diseases found on both warm and cool-season turfgrass species around the world. *Sclerotinia homoeocarpa*, the fungal pathogen that causes dollar spot, is genetically classified into groups based on host species. Previous studies have shown various methods to reduce disease pressure without synthetic fungicides. Dollar spot is suppressed with 48.8 kg ha^{-1} ferrous sulfate heptahydrate in field studies. Lab research has shown 100 to 1,000 mg kg^{-1} ferrous sulfate concentrations in agar to directly inhibit *Sclerotinia homoeocarpa* mycelial growth *in vitro*. Dollar spot response to varying levels of ferrous sulfate is not clearly defined. Response of isolates by host and previous management history to ferrous sulfate has not been investigated. Our research explored five field rates and five concentrations of ferrous sulfate against dollar spot infection and *Sclerotinia homoeocarpa* growth, respectively. Field rates included 0, 4.88, 24.4, 48.8, and 97.6 kg ha^{-1} ferrous sulfate (heptahydrate 20% Fe, Valudor Products Inc.). Our data indicate a nonlinear relationship between ferrous sulfate rate and dollar spot suppression with the most rapid disease reduction occurring below 24.4 kg ha^{-1}. Our model estimates 26.4 kg ferrous sulfate ha^{-1} is required to suppress dollar spot populations by 50%. To expand on previous *in vitro* studies, dollar spot isolates were collected from both cool- and warm-season turf species in areas with no historical fungicide use and extensively treatment. Modified potato dextrose agar was amended with concentrated of 0, 200, 400, 600, and 800 mg/ kg^{-1} of ferrous sulfate. Our data show that the effective concentration required to suppress *S. homoeocarpa* growth by 50% ranges from 480 to 720 mg L^{-1}. Isolates collected from cool-season grasses grew more aggressively than isolates collected from warm-season grasses. More importantly, we concluded the ferrous sulfate activity against *S. homoeocarpa* was dependent on previous management inputs. This research provides more refined guidelines to turf managers on dollar spot suppression with ferrous sulfate.

Introduction

Dollar spot is a chronic problem that affects both warm- and cool-season grasses on golf courses throughout the world (Burpee et al, 1997). It is caused by the fungal pathogen *Sclerotinia homoeocarpa* F.T. Bennett (Monteith and Dahl, 1932). Exploration of the phylogentic makeup of *S. homoeocarpa* has revealed two distinct groups; those isolated from cool-season grasses and those isolated from warm-season grasses (Aynardi et al., 2016).

There are many strategies that turf managers implement to control dollar spot. These include: nitrogen fertilization, shade reduction, increasing air circulation, reducing leaf wetness period, resistant cultivars, and fungicide applications (Walsh et al, 1999). Judicious use of fungicides which target the pathogen is the most common and effective practice. Multiple fungicide applications are required throughout the growing season to suppress disease to acceptable levels which increases the chance for resistance (Walsh et al., 1999). More money is spent each year to manage dollar spot than to control any other turf disease (Goodman and Burpee, 1991).Cost, use rates, fungicide resistance, and governmental restrictions have led scientists to explore alternative options to control this disease (Latin, 2012). Several cultural strategies have been developed to help suppress dollar spot populations. Practices targeting moisture removal from the leaf surface can be effective for reducing the disease (Williams et al., 1996 and Ellram et. al, 2007). A heavy thatch layer can prevent adequate moisture from reaching the roots and keep more water on the surface (Couch, 1995). Adequate nitrogen fertilization has been shown to reduce dollar spot development (Markland et al., 1969). In recent years, research utilizing ferrous sulfate for pathogen suppression in turf systems has been tested (Mattox et. al, 2015 and McCall et. al, 2016).

The use of iron for pathogen suppression was first noted by Forsyth et. al (1957). In this study, authors discovered a reduction in rust (*Puccinia graminis* Pers.) hyphae on wheat plants using ferrous sulfate. The use of ferrous sulfate to reduce turfgrass pests has been reported, but without validated research until recently (Graham, 1983 and Arthur, 2003). Subsequently, Microdochium patch incidence decreased with increasing rates of ferrous sulfate (Mattox et al, 2015). Ferrous sulfate reduced dollar spot in one study but the product’s elemental components of iron and sulfur were inconsistent and ineffective, respectively (McCall et. al, 2016).The authors also reported ferrous sulfate to be directly fungitoxic to *S. homoeocarpa* at concentrations between 100 and 1000 mg L^{-1} in amended potato dextrose broth.

Although research has shown that ferrous sulfate can suppress dollar spot populations, more information is needed to make applications more effective. Previous studies have only tested 48.8 kg ha^{-1} of ferrous sulfate for dollar spot suppression (McCall et. al, 2016). It is unknown how the dollar spot pathogen responds to ferrous sulfate rates beyond what is previously reported. Mycelial growth inhibition with ferrous sulfate reported by the authors was conducted with an isolate collected from an intensively managed creeping bentgrass stand that had been routinely treated with fungicides. More research is needed to determine if *S. homoeocarpa* isolate diversity may play a role in suppression.

Therefore, our research objectives were to 1) further define the relationship between ferrous sulfate rate and dollar spot incidence on creeping bentgrass in the absence of fungicide applications, and 2) determine *S. homoeocarpa* isolate response to ferrous sulfate based on original collection host and previous fungicide inputs.

Materials and Methods

Field evaluation of ferrous sulfate. Field trials were conducted at two creeping bentgrass sites in Blacksburg, VA, between June and August of 2016 and 2017. Trials were conducted on a mature '007' creeping bentgrass putting green at the Virginia Tech Turfgrass Research Center, and on a one-year-old '96-2' creeping bentgrass fairway (2016) and established 'L-93' creeping bentgrass fairway (2017) at the Glade Road Research Facility. Both established locations had a history of heavy dollar spot infestation. Dollar spot pressure was expected to be moderate to high on the '96-2' fairway, based on previous performance (cite NTEP data). The '96-2' fairway location suffered severe drought damage late in 2016 and had to be re-established. This necessitated moving the fairway trial location in 2017. The putting green was built to USGA specifications (90% sand, 10% peat moss) and mowed five times a week at 3.2 mm with clippings removed. Fairways were established on a silt loam and mowed three times per week at 16.5 mm. The green plot area was core aerified and sand top dressed in the spring and fall, removing approximately 15% surface area for the year. Both the green and fairway were irrigated as needed to prevent visual signs of wilt. For all greens trials, nitrogen was applied biweekly following trial initiation in the form of urea at a rate of 7.3 kg N ha^{-1} totaling 43.8 kg N ha^{-1} yr^{-1}. Granular slow-release nitrogen sources were used in the spring and fall following aerification.

Five treatments of ferrous sulfate (heptahydrate 20% Fe, Valudor Products Inc.) were arranged in a randomized complete block design over four replications to evaluate efficacy against dollar spot. Rates tested were determined to further define effective concentrations needed to suppress dollar spot by previous research that tested 48.8 kg ha^{-1} (McCall et al., 2017). All treatments were applied bi-weekly as suspension in a water carrier with a CO_2-pressurized (275.6 kPa at 814 L ha^{-1} and 407 L ha^{-1}) backpack sprayer through TeeJet TTI 11004VP nozzles. Treatments included 0 kg ha^{-1}, 4.88 kg ha^{-1}, 24.4 kg ha^{-1}, 48.85 kg ha^{-1}, and 97.6 kg ha^{-1}. Individual plots measured 1.8m x 1.8m.

Counts of individual dollar spot infection centers, visual estimates of percent disease, and visual turf quality were collected weekly for 12 weeks following trial initiation. Dollar spot count data were collected by counting the total number of infection centers per plot. A 3 x 3 grid with 296 squares was used periodically throughout the trials to maintain alignment throughout the season. Turf quality were based on a 1-9 visual estimation scale with 1 = dead, poor quality turf, 6 = minimally-acceptable turf, and 9 = healthy, high quality turf (Morris, 2002).

Dollar spot counts and percent disease were transformed using the area under disease progress curve (AUDPC) to quantify dollar spot intensity over the duration of the study (Madden et al., 2007). The AUDPC values were calculated using the formula:

$$AUDPC = \left\{ \sum_{i=1}^{n-1} \left[\left(y_i + y_{i+1} \right) / 2 \right] * \left(t_{i+1} - t_i \right) \right\}$$

Turf quality was transformed using a standardized AUTQPC to represent daily estimates throughout the duration of the study. Data were subjected to analysis of variance (ANOVA) using the PROC NLIN procedure in SAS, version 9.1 (SAS Institute, Cary, NC) and included rate, year, location, and their interactions. Non-linear Gauss-Newton hyperbolic regression was performed with estimated parameters of a = 89.46 and i = 4.3. The rate needed to reduce infection center counts by 50% (IR_{50}) was determined using the Gauss-Newton model. Visual estimates of percent dollar spot and turf quality were compared as dependent variables against infection center counts using general linear regression.

***In-vitro* response to ferrous sulfate.** The effect of ferrous sulfate on *S. homoeocarpa* mycelial growth was studied *in vitro* with four fungal isolations representing two grass types and two management intensities. Potato dextrose agar (PDA, Bacto, Difco Laboratories, Detroit, MI) was prepared at ¼ strength and supplemented with granulated agar, as described by Amaradasa et. al (2014), and amended with five concentrations of ferrous sulfate; 0, 200, 400, 600, and 800 mg kg^{-1}. Treatments of a 5 x 2 x 2 factorial design were completely randomized over five replications and repeated once.

Isolated cultures of *S. homoeocarpa* were collected from randomly selected locations across Virginia. Symptomatic tissue was collected from warm- and cool-season turf golf courses, with highly variable management intensities. Infected leaf samples were surface sterilized with 10% bleach (6% sodium hypochlorite) for 30 seconds, rinsed three times in sterile deionized water, and blotted dry on sterile paper towels. Leaf clippings were placed on ¼ strength PDA where mycelia were observed for growth and hyphal tips were transferred to new petri dishes to eliminate contamination. Colony morphology of pure colonies consistent with *S. homoeocarpa* (Smiley et al., 2005) were selected for further investigation. A total of 18 isolates were collected from all sites. Of these isolates collected, 5 came from intensively managed cool-season turf, 4 came from low-input cool-season turf, 5 came from intensively managed warm-season turf, and 4 came from low-input warm-season turf. Four isolates were selected to represent low and high management intensity collected from warm-season and cool-season grasses. No fungicide isolates (NFI) were cultured from low input turf areas that had never been treated with fungicides, whereas routine fungicide isolates (RFI) were obtained from high input turf that had received intensive historical fungicide use. The selected low input warm- and cool-season cultures were isolated from bermudagrass (WO13) and perennial ryegrass (GR7) general-use areas, respectively. Cultures from intensively managed warm- and cool-season grasses came from a bermudagrass fairway (IC15) and a creeping bentgrass putting green (TRC4), respectively. Isolates were stored on PDA and transferred to new plates as needed to maintain viability.

Prepared ¼ PDA material was added to distilled, deionized water, stirred continuously for five minutes, and autoclaved for sterilization. Each concentration of ferrous sulfate (heptahydrate 20% Fe, Valudor Products Inc.) was added to PDA during cooling and stirred for five minutes, with 0.10 mL of lactic acid added to each suspension to prevent contamination. The suspension pH was adjusted to 6.5 using lactic acid using a pH meter (VWR Scientific Products SR 601C, Radnor, PA). A 5 mm disk of actively growing mycelia was placed upside down on amended PDA. Petri plates were incubated in darkness at ambient air temperature (20-22°C) on a laminar flow hood bench. One PDA plate of each amended ferrous sulfate concentration was used to monitor microbial contamination. No contamination was observed during the trial periods. The mycelial growth of each plate was averaged from two perpendicular measurements after 72hr incubation.

Results and Discussion

Field evaluation of ferrous sulfate. The linear relationship ($P < 0.0001$, $r^2 = 0.99$) between dollar spot infection center counts and visual estimates of percent disease was highly significant (Figure 1).

Therefore, only data based on infection-center counts will be presented to explain the influence of ferrous sulfate rates on dollar spot progression. However, presenting this clear relationship may be useful for future researchers, as estimations of disease pressure is more efficient than counting individual infection centers. We were unable to find documentation of this relationship from previous literature. Horvath and Vargas (2005) reported that digital image analysis was not closely related to dollar spot infection center counts. The authors attributed this to variability in infection center size that is not considered when counting foci. Digital image analysis was used as a representation of the percent area infested with dollar spot. While the size of infection centers varied in our research, the average diameter was approximately 4-cm wide with heavy disease pressure (data not shown). Disease pressure in our study was approximately 10x higher than in data presented by Horvath and Vargas (2005). Standard error is decreased as sample size increases (McDonald, 2014), and thus, likely explains the higher correlation between cover of diseased turf and infection center counts in our research than previously reported. The use of visual estimations to quantify percent disease may not always be explained as clearly as presented in this manuscript because of intra-rater and inter-rater variability. Nutter et al., (1993) presented greater variability in dollar spot assessments by individual raters and in ratings from one individual over time than objective radiometric measurements. Both rater bias and rater experience can impact accuracy and precision of visual assessments of percent disease ratings (Madden et. al, 2007). We recommend a baseline relationship to be established for individual raters over time when using visual estimates of percent disease to represent overall disease pressure.

Although both main effects and interactions of the random variables year and location were significant ($P \leq 0.04$), year and location did not interact with ferrous sulfate rate ($P \geq 0.06$, Table 1) to influence percentage reduction in seasonal AUDPC based on infection-center counts. Therefore, the effect of ferrous sulfate rate on AUDPC reduction was subjected to hyperbolic function pooled across years and locations (Figure 2). The significance of year and location effects for both AUDPC reduction and IR_{50} values (Table 1) account for variable magnitude of disease pressure between sites (data not shown). Differences in disease pressure observed between years may be due to thatch accumulation (Warnke, 2003). In 2016, fairway trails were established on ’96-2’ creeping bentgrass and on ‘L-93’ creeping bentgrass in 2017. The fairway site used in 2016 was a new establishment with only one season of growth. The fairway utilized for this research in 2017 has approximately 10 years of growth and lacked prior thatch management practices. To normalize for these differences in disease pressure between locations and years, seasonal AUDPC values in treated plots were converted to a percentage reduction based on the seasonal AUDPC in non-treated plots of the same year, location, and replicate. The result was a consistent trend in the response of AUDPC reduction to ferrous sulfate rates (Figure 2).

The hyperbolic trend with an estimated *I* of 187.6 showed that ferrous sulfate quickly reduced seasonal AUDPC of dollar spot by approximately 50% and then continued to slowly approach an asymptote of 89.5% disease reduction (Figure 2). Based on the predicted trend, seasonal disease reductions of 25, 50, and 75% would require 8.0, 26, and 100 kg ha^{-1} ferrous sulfate, respectively. Applications of 97.6 kg ha^{-1} ferrous sulfate caused creeping bentgrass phytotoxicity in our study when applied during high air temperatures and is not recommended (data not shown). Although little research has demonstrated effects of ferrous sulfate on fairways and greens, peer-reviewed research by McCall et al, (2016) supports our findings that ferrous sulfate applied to creeping bentgrass putting greens can reduce dollar spot incidence. Our research provides evidence that ferrous sulfate rates can be decreased by 50% from previously reported amounts with only an approximated 10% loss in efficacy. Earlier work with other iron chelates and ferrous sulfate only observed dollar spot control as general observations to existing research (Graham, 1983)

***In-vitro* response to ferrous sulfate.** Results of the *in vitro* test of ferrous sulfate effects on *S. homoeocarpa* radial mycelial growth as affected by isolate type and ferrous sulfate concentration was not dependent on trial (Table 2). All interactions with trial were insignificant for radial mycelial diameter ($P \geq 0.3966$) and for relative diameter ($P \geq 0.0811$, Table 2). Generally, isolates collected from cool-season turfgrass tended to grow more rapidly than those collected from warm-season turfgrass (Figure 3). To more clearly measure the influence of ferrous sulfate on radial growth while controlling for error associated with host-specific virulence, data were converted to a percentage growth relative to the nontreated check. This transformation was effective as the previously-significant main effect of isolate host ($P = 0.0149$) was no longer significant when data were converted to relative colony diameter ($P = 0.8204$, Table 2). When this research was conducted, differences in *S. homoeocarpa* virulence had been empirically observed and marginally related to host species but specific relationships had not been elucidated. Subsequent research by Aynardi (2016) reported *S. homoeocarpa* to be both polymorphic and polyphenic. Of the two isolate types described by Aynardi (2016), Type I isolates were characterized by increased virulence compared to Type II isolates. Type 1 isolates are host adapted in warm- and cool-season turfgrasses while type 2 isolates are found only on warm season turfgrasses (Aynardi et al., 2016). In the current study, isolates from cool-season turf were consistently more virulent than those from warm-season turf and are presumed to be Type 1. Isolate virulence has been shown to influence disease management efforts in other studies (Hsiang et al., 1998).

The interaction of turfgrass host by history of fungicide input by ferrous sulfate concentration was significant for both radial mycelial growth and relative growth percentages ($P \leq 0.0092$, Table 2). Regressions relating ferrous sulfate concentration to percentage relative colony diameter are presented with respect to cool- and warm-season turf hosts separately by RFI and NFI sites and by inputs separately by isolates taken from cool- and warm-season turfgrasses (Figure 4). Despite a significant three-way interaction ($P = 0.0092$, Table 2), relative colony diameter response to ferrous sulfate concentration was generally consistent with respect to isolate host (Figure 4A, 4B). Slight differences in relative colony diameter response to ferrous sulfate between isolates collected from cool- (Figure 4A) and warm-season (Figure 4B) turfgrass contributed to the significant interaction. Since neither predicted or observed values varied by more than 5% in relative colony diameter, the influence of isolate host appears to be of minor biological significance (Figure 4A, 4B). Historical fungicide inputs, however, interacted more strongly with ferrous sulfate rates to influence relative colony diameters. Evidence of this influence is found in the generally linear decline in relative colony diameter regardless of isolate host for NFI isolates (Figure 4A) compared to a curvilinear response where relative colony diameters declined more slowly for RFI isolates (Figure 4B). Differences between historical fungicide inputs with respect to relative colony diameter can be more easily compared in Figures 4C and 4D. For every 100 mg L^{-1} increase in ferrous sulfate concentration, relative colony diameter decreased approximately 9% regardless of isolate host for NFI isolates (Figures 4C, 4D). A more curvilinear decay in relative colony diameter with increasing ferrous sulfate concentration was evident for RFI isolates (Figures 4C, 4D). Thus, RFI isolates required 175 and 100 mg L^{-1} more ferrous sulfate in cool- and warm-season turfgrasses, respectively to reduce relative colony diameters by 50% compared to NFI isolates (data not shown).

Results of this study are similar to that of Reams et al. (2013) in that increasing ferrous sulfate concentration decreases mycelial growth, and indicates a direct fungitoxic effect. Thus, field-applied ferrous sulfate is likely preventing the pathogen from infection on the leaf surface in addition to potential plant health benefits (Venu et al., 2009). Although information is not available with respect to ferrous sulfate, *S. homoeocarpa* has routinely demonstrated an ability to adapt to turfgrass management inputs including fungicides (Hsiang at al., 1998) and cultural practices (Jo et al., 2008). In addition to fungicide use, the RFI sites used in this study can also be characterized as historically receiving greater variability and frequency of general management inputs such as mowing, fertility, herbicides, insecticides, and aeration. Data from the current study suggest that isolates from sites exposed to fungicide and/or increased management inputs require 1.3 to 1.4 times more ferrous sulfate to reduce growth 50% (Figure 4). Cross resistance of *S. homoeocarpa* to multiple fungicides has been documented (Jo et al., 2008) but relationships between prior fungicide exposure and adaptation to other cultural practices, or ferrous sulfate specifically, has not been reported.

Conclusion

The results from these studies indicate that field-applied ferrous sulfate reduces dollar spot in managed creeping bentgrass greens and fairways. Approximately 26 kg ha^{-1} ferrous sulfate is needed to reduce seasonal AUDPC of dollar spot by 50% and increasing ferrous sulfate rate beyond that point may be met with diminishing returns of disease control value. *In*-vitro studies confirm that ferrous sulfate imparts a direct fungitoxic effect on *S. homoeocarpa* mycelial growth. We can further support work by Aynardi (2016) that suggest variable virulence of *S. homoeocarpa* isolates can be found in cool- and warm-season turfgrass but we can add that inherent virulence has minimal influence on mycelial control by ferrous sulfate. Isolates from sites characterized by routine fungicide use may require up to 1.5 times more ferrous sulfate to achieve comparable mycelial growth inhibition as isolates from sites that have never received fungicide.

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Appendix: Tables and Figures

Table 1: Analysis of variance for percentage reduction relative to nontreated plots of area under the disease progress curve (AUDPC) based on dollar spot infection centers 3.2 m^{-2} assessed weekly twelve times and of ferrous sulfate concentrations needed to reduce AUDPC 50% (IR_{50}).

Source		**Reduction of AUDPC#**		**IR_{50}**	
	df	**F Value**	**Pr > F**	**F Value**	**Pr > F**
replicate	3	3.24	0.0285	31.72	<.0001
year	1	26.27	<.0001	483.79	<.0001
location	1	9.63	0.0030	99.69	<.0001
location*year	1	4.37	0.0411	19.94	<.0001
$FeSO_4$ rate	4	81.62	0.0004		
year* $FeSO_4$ rate	4	2.39	0.0616		
location* $FeSO_4$ rate	4	0.63	0.6397		
location*year* $FeSO_4$ rate	4	1.79	0.1437		

[a]This field study was conducted as a randomized complete block design and repeated in time and space with random variables replicate, location, and year. Treatments included five rates of ferrous sulfate. All effects associated with random variables were tested with experimental error. All other effects or interactions were tested with the mean square of its interaction with the random variable.

Table 2: Analysis of variance of *in vitro Sclerotinia homoeocarpa* growth following 3 d incubation in 0.25-strength potato dextrose agar amended with various ferrous sulfate concentrations and assessed as original diameters and percentage of colony diameter relative to non-treated plates.

		Diameter (mm)			**Relative diam. (%)**	
Source[a]	**df**	**F Value**	**Pr > F**		**F Value**	**Pr > F**
replicate	4	1.82	0.1272		0.63	0.6406
trial	1	3.48	0.0641		5.59	0.0193
host	1	1828.52	0.0149		0.08	0.8204
input	1	178.98	0.0475		1213.87	0.0183
concentration	4	3363.46	<.0001		2719.75	<.0001
host*input	1	225.92	0.0423		4.39	0.2834
host*concentration	4	186.36	<.0001		26.15	0.0040
input*concentration	4	173.81	<.0001		120.29	0.0002
host*input*concentration	4	26.76	0.0038		16.71	0.0092
trial*host	1	0.72	0.3966		3.08	0.0811
trial*input	1	0.22	0.6394		0.17	0.6797
trial*concentration	4	0.57	0.6865		0.57	0.6876
trial*host*input	1	0.68	0.4108		0.08	0.7777
trial*input*concentration	4	0.41	0.7996		0.38	0.8262
trial*host*concentration	4	0.57	0.6842		0.62	0.6474
trial*host*input*concentration	4	0.65	0.6301		0.40	0.8112

[a]This laboratory study was conducted as a completely randomized design and repeated in time with random variables replicate and trial. Treatments were arranged in a 2x2x5 factorial design. Isolates were collected at sites that routinely or never received fungicides (input), and from cool-season or warm-season turfgrass species (host). Inoculum from isolates was plated in agar containing ferrous sulfate at 5 levels between 0 and 800 ppm (concentration). All effects associated with random variables were tested with experimental error. All other effects or interactions were tested with the mean square of its interaction with trial.

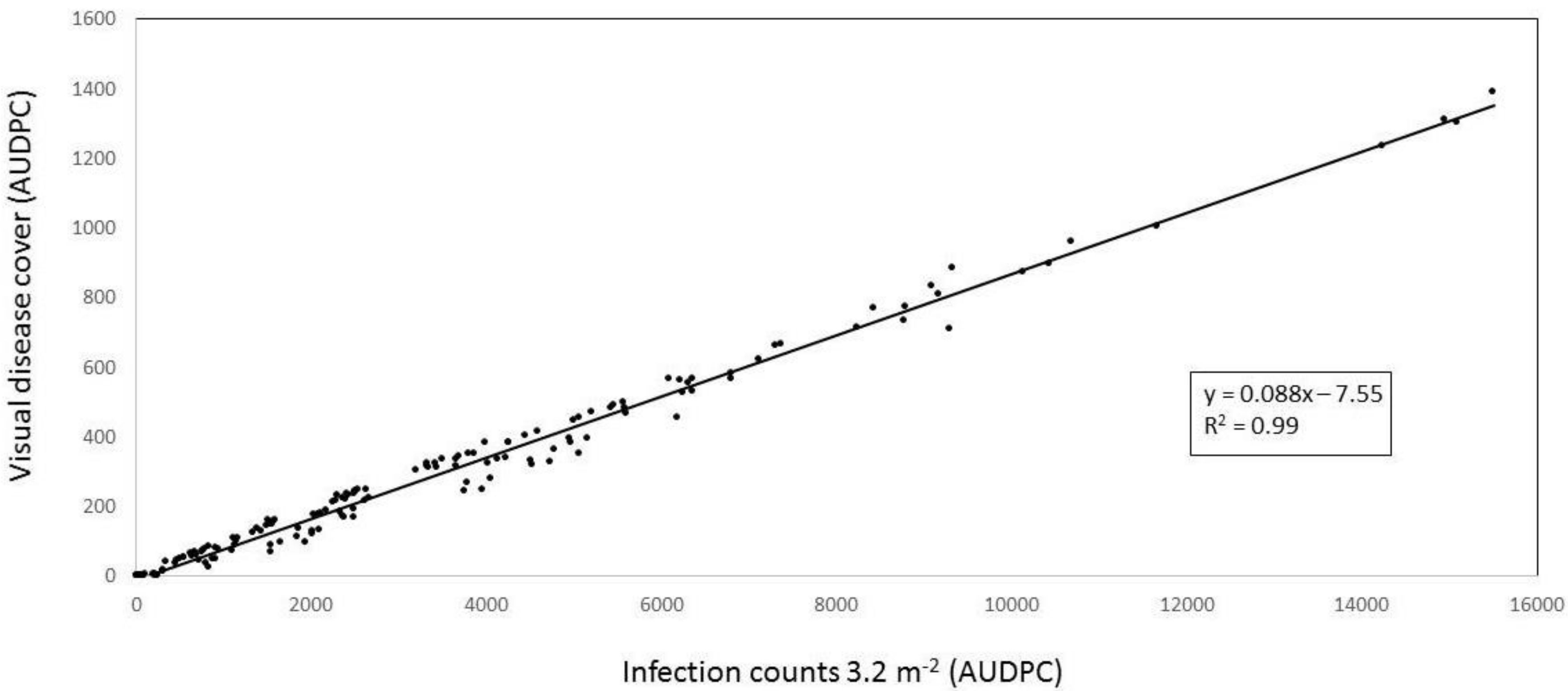


Figure 1: Correlation of area under the disease progress curve (AUDPC) for 12 weekly assessments of infection counts per plot versus visually-estimated percentage diseased turf for a total of 160 observations.

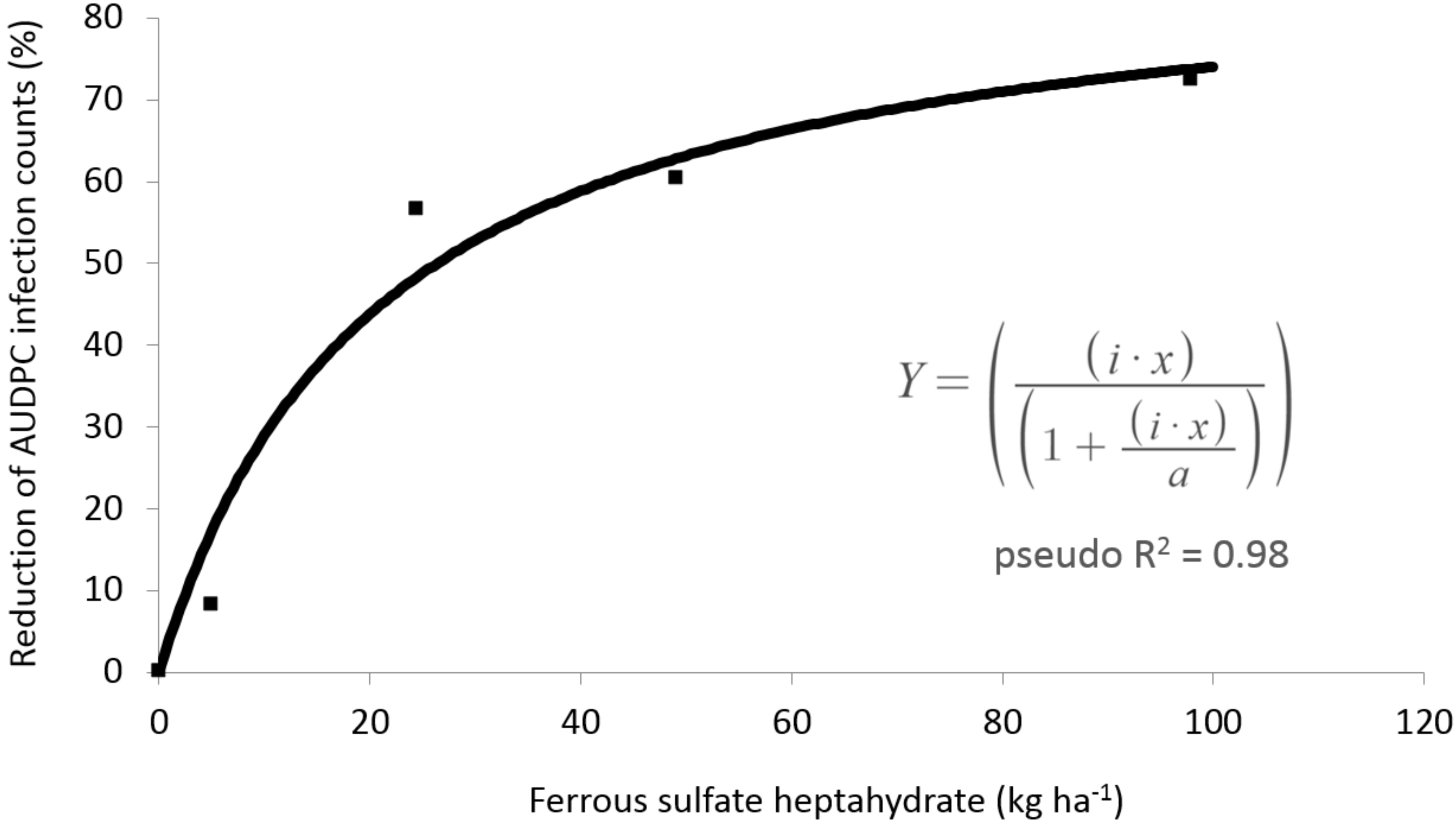
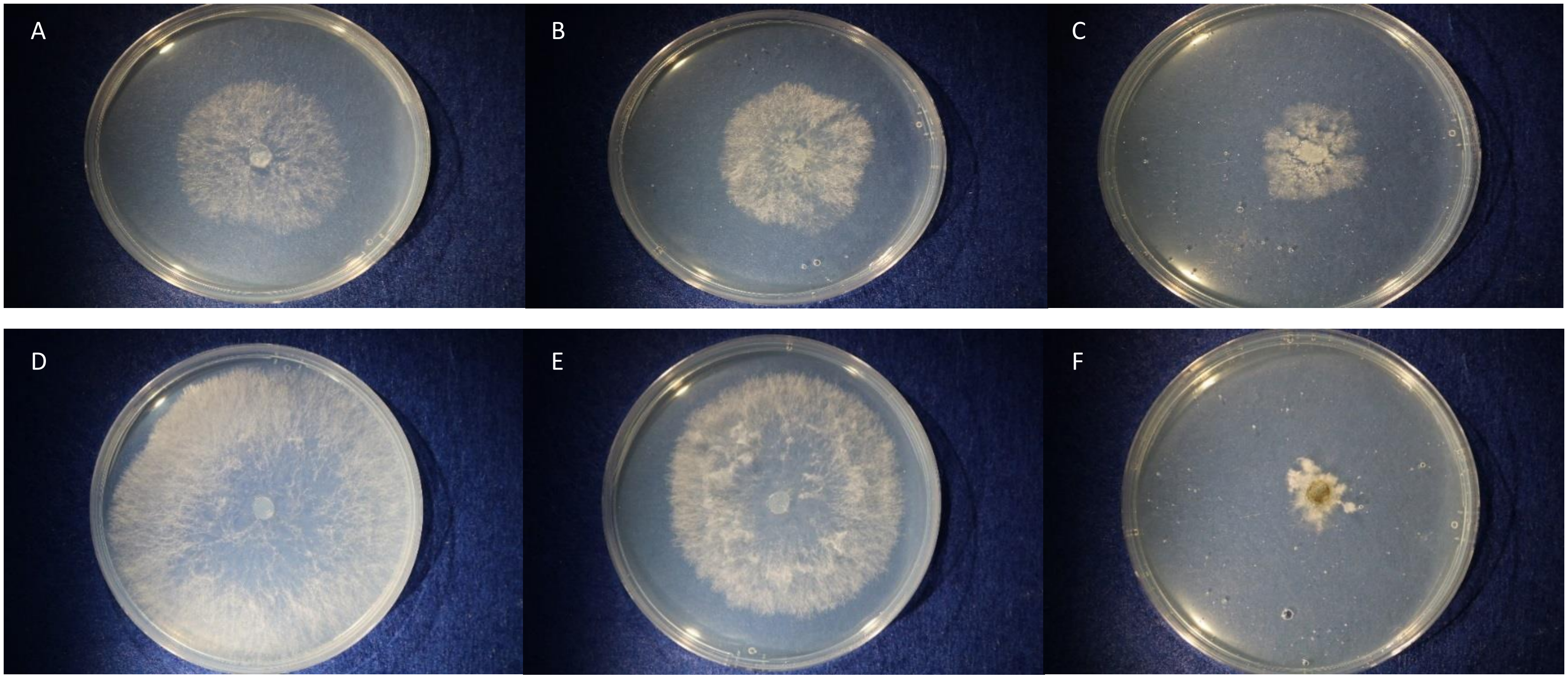


Figure 2: Influence of ferrous sulfate rate on reduction in area under the disease progress curve (AUDPC) relative to the nontreated check based on dollar spot infection centers 3.2 m^{-2} assessed weekly twelve times.

Figure 3: Colony growth of 3-d-incubated *Sclerotinia homoeocarpa* isolated from warm-season (A, B, C) or cool-season (D, E, F) turfgrass and exposed to 0 (A, D); 400 (B, E); or 800 (C, F) mg L^{-1} ferrous sulfate in 0.25 strength potato dextrose agar. Plates shown represent random isolates from sites that historically received fungicide treatments.



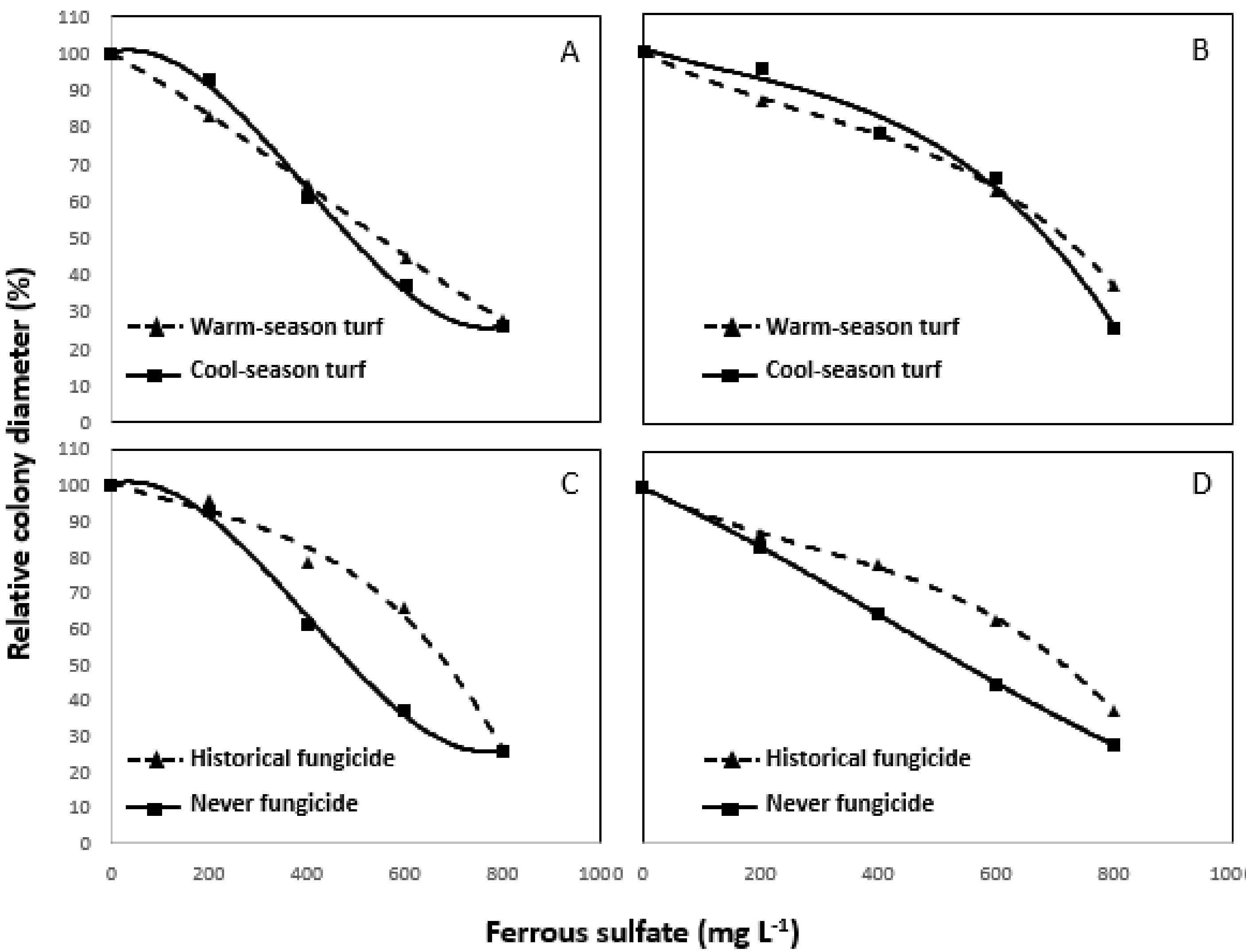


Figure 4: Response of relative *Sclerotinia homoeocarpa* colony diameter to ferrous sulfate concentration as influenced by turfgrass host in sites that received historical fungicide use (A) and sites that never received fungicide (B) and as influenced by historical fungicide use when isolates were collected from warm-season (C) or cool-season (D) turf.