AN ABSTRACT OF THE DISSERTATION OF

Clint Michael Mattox for the degree of Doctor of Philosophy in Horticulture presented on February 26, 2020.

Title: Suppression of Microdochium Patch on Annual Bluegrass Putting Greens using Iron Sulfate, Phosphorous Acid, Sulfur, and Mineral Oil

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Managing Microdochium patch on intensively manicured annual bluegrass putting greens is a challenge for turfgrass professionals in cool-humid climates similar to the Pacific Northwest. Fungicides are the predominant means to mitigate damage caused by this fungal pathogen, however pesticide restrictions are making it even more challenging to suppress Microdochium patch. Five field experiments, two growth chamber experiments, and two *in vitro* experiments were carried out to explore the use of iron sulfate heptahydrate, phosphorous acid, sulfur, and mineral oil on the suppression of Microdochium patch and on turfgrass quality. Mineral oil combined with either sulfur or phosphorous acid suppressed Microdochium patch, although combinations of mineral oil and sulfur reduced turfgrass quality, especially in the winter months. This reduction provides evidence that mineral oil and sulfur combinations should be avoided under similar conditions. Eliminating mineral oil applications in the winter months and replacing these applications with a sulfur and phosphorous acid combination suppressed Microdochium patch and mitigated damage although a temporary loss of turfgrass quality was still observed. Applying iron sulfate heptahydrate every two weeks suppressed Microdochium patch but

resulted in suboptimal turfgrass thinning. Increasing the application interval of iron sulfate heptahydrate beyond two weeks decreased the level of Microdochium patch suppression observed. Increasing the water carrier volume of iron sulfate heptahydrate applications resulted in less abiotic damage quantified by having higher green cover percentages and these higher carrier volumes did not have a negative impact on Microdochium patch suppression. No benefit in Microdochium patch suppression was observed when adding iron sulfate heptahydrate to phosphorous acid applications compared to phosphorous applications alone, although turfgrass quality was improved when phosphorous acid was used in combination with some rates of iron sulfate heptahydrate. Both iron sulfate heptahydrate and phosphorous acid applications reduced the turfgrass surface pH for up to 17 days post application. Subsequent growth chamber and *in vitro* studies suggested that a reduction in pH was not solely responsible for the suppression in Microdochium patch by iron sulfate heptahydrate applications. These studies have demonstrated that multiple approaches of suppressing Microdochium patch are available to turfgrass managers and that future research is warranted in using these techniques as a part of an integrated pest management plan.

Suppression of Microdochium Patch on Annual Bluegrass Putting Greens using Iron Sulfate, Phosphorous Acid, Sulfur, and Mineral Oil.

by

Clint Michael Mattox

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APPROVED:

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Clint Michael Mattox, Author

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CONTRIBUTION OF AUTHORS

This dissertation consists of multiple chapters written specifically to be submitted to the Agronomy Journal with the exception of chapter 4 that was published as a brief for the Crop Forage and Turfgrass Management Journal. The contributing authors for the chapters are as follows:

Chapters 1, 2 and 5: Mike Dumelle assisted with statistical analyses and interpretation

Chapter 3: Alec Kowalewski and Brian McDonald co-authored this publication

Chapter 4: Alec Kowalewski and Brian McDonald co-authored this publication

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Introduction

Microdochium patch is a turfgrass disease caused by the fungal pathogen *Microdochium nivale* (Fries) Samuels and I.C. Hallett (teleomorph: *Monographella nivalis* (Schaffnit) E. Müller). This disease occurs in cool and humid regions like the areas along the coast of the Pacific Northwest of North America (Smiley et al., 2005; Vargas, 2005). In the United Kingdom, Microdochium patch is reported to be a concern on the majority of golf courses (Mann and Newell, 2005). Annual bluegrass (*Poa annual* L.) is particularly prone to Microdochium patch (Smiley et al., 2005) and in the Pacific Northwest, annual bluegrass is the most common turfgrass grown on golf course putting greens (Lyman et al., 2007) even though it is seldomly the grass that was originally established. Some creeping bentgrass (*Agrostis stolonifera*) cultivars have demonstrated a decrease in susceptibility to Microdochium patch (NTEP, 2020), however annual bluegrass outcompetes creeping bentgrass in cool and humid areas, especially during the winter months where there is limited snow or freezing conditions (Vargas and Turgeon, 2004). Efforts to breed annual bluegrass and potentially release cultivars less susceptible to Microdochium patch has been a challenge because the perennial phenotype of annual bluegrass is reported to be unstable (La Mantia and Huff, 2011). The reliance on the use of herbicides and growth regulators to eliminate annual bluegrass is also not without risk because of the chances of herbicide resistance (Cross et al., 2015) and restrictions in certain areas on how herbicides, if any, can be used (Ministère de l'agriculture et de la péche, 2006). In the Pacific Northwest, the elimination of annual bluegrass from creeping bentgrass putting greens has not been met with success, therefore annual bluegrass remains the predominant turfgrass on putting greens in these areas (Lyman et al., 2007) and Microdochium patch remains a major concern (Vargas, 2005).

Microdochium patch can occur year-round in cool-humid areas (Smiley et al., 2005), although this disease occurs most commonly when temperatures are between 8 and 17°C and

humidity is ≥ 90% for 20 hours or more (Dwyer et al., 2017). Microdochium patch symptoms first appear as small orange brown patches less than 5 cm in diameter and can enlarge to patches generally less than 20 cm in diameter (Smiley et al., 2005). In order to meet expectations of turfgrass managers and the golfing public (Walsh, 2005), fungicides are predominantly used to manage Microdochium patch on high value areas, such as golf course putting greens (Golembiewski and McDonald, 2011; Aamlid et al., 2015). In most regions of the United States, many fungicides are available to manage Microdochium patch with multiple families of chemistries reported to provide "excellent" control (Clarke et al., 2019) allowing for the possibility for fungicide rotations. In spite of the number of chemistries available, Microdochium patch occurs for more than half of the year in cool and humid areas that experience above freezing conditions throughout the winter. The frequent use of fungicides in these areas have led to reports of fungicide resistance or reduced efficacy. Resistance has been documented for fungicides belonging to the class of dicarboximides (Chastagner and Vassey, 1982; Pennucci et al., 1990; Gourlie and Hsiang, 2017), quinone outside inhibitors (Walker et al., 2009) and methyl benzimidazole carbamates (Huth and Schlösser, 1980; Tanaka et al., 1983; Larson, 1983).

Reports of fungicide resistance (Allan-Perkins et al., 2019) and limits to fungicide chemistries permitted in certain areas (Ministère de l'agriculture et de la péche, 2006; Christie, 2010; San Francisco, 2019) is making it more challenging for golf course superintendents to manage Microdochium patch. For these reasons, alternatives to fungicides are desired to mitigate fungicide resistance and to follow legal restrictions in these areas. Potential options that may be permitted where pesticide restrictions occur are organically registered fungicides such as mineral oils or sulfur (Portland, 2020; OMRI 2020a, 2020b), cultural practices such as rolling, or

fertilizer products such as iron sulfate heptahydrate. Another potential option in areas where chemical fungicides are restricted is the use of phosphorous acid products.

The mineral oil Civitas Turf Defense is registered as an organic fungicide (OMRI, 2020a) and previous studies using this mineral oil to suppress Microdochium patch have shown promising results. Mineral oil has been shown to suppress Microdochium patch on annual bluegrass research greens in Western Oregon (Mattox et al., 2020) and on a variety of mixed turfgrass stands on putting greens in Scandinavia (Aamlid et al., 2018). There is evidence that the mineral oil induces systemic resistance in creeping bentgrass and also has some fungistatic activity against *M. nivale* (Cortes-Barco et al., 2010a) providing some explanation as to why the mineral oil may be suppressing Microdochium patch under field conditions. One concern regarding the frequent use of mineral oils in Western Oregon was a loss in turfgrass quality caused by turfgrass thinning that was especially evident when combined with rolling (Mattox et al., 2020). The cause of thinning has not yet been elucidated, however stomatal occlusion by the mineral oil may result in reduced gas exchange (Kreuser and Rossi, 2014) and a reduction in the plants ability to perform photosynthesis.

Some sulfur products are registered as organic fungicides (OMRI, 2020b) and previous studies using these products to suppress Microdochium patch have shown promising results. Sulfur is a fungicide that has been used for plant pest control for over 2000 years (Beckerman, 2008; Smith and Secoy, 1975) and is considered to have multi-site activity with a low risk of resistance (Hewitt, 1998; Fungicide Resistance Action Committee, 2019). There are no known reports of field resistance to sulfur applications on any crops (Cooper and Williams, 2004). Research in Western Oregon demonstrated that 12.2 kg S ha^{-1} applied every two weeks suppressed Microdochium patch on annual bluegrass putting greens, with an even greater

suppression of Microdochium patch observed when sulfur applications were combined with 3.7 kg phosphorous acid ha^{-1} (Mattox et al., 2020). A concern that remains regarding the use of frequent sulfur applications is the potential increased risk of anthracnose (*Colletotrichum cereale*) in the summer (McDonald et al., 2018).

Phosphorous acid products are often marketed as fertilizers, although these phosphonate products (Guest and Grant, 1991) are reported to have fungicidal characteristics (Vincelli and Dixon, 2005) and are considered to be a low-risk pesticide regarding environmental impact and human safety (Latin, 2011). Phosphorous acid is phloem-mobile systemic (Ouimette and Coffey, 1990) and the mode of action of phosphorous acid is considered to induce host plant resistance (Fungicide Resistance Action Committee, 2019). There is evidence that phytoalexin production measured as total phenolic compounds increases when phosphorous acid is applied to annual bluegrass and creeping bentgrass, leading to the suppression of Microdochium patch (Dempsey, 2016). Furthermore, experiments have shown that phosphorous acid applications led to a reduction in *M. nivale* conidial germination and that hyphae expressed morphological deformities suggesting a fungistatic effect (Dempsey et al., 2012). Phosphorous acid has been shown to suppress Microdochium patch on velvet bentgrass (*Agrostis canina* L. ssp. canina) and annual bluegrass in Ireland when applied at 3.8 kg H_3PO_3 ha^{-1} (Dempsey et al., 2012) and on annual bluegrass in Western Oregon when applied at 3.7 kg H_3PO_3 ha^{-1} (Mattox et al., 2020).

Iron sulfate heptahydrate is a fertilizer that has been shown to enhance turfgrass color (Yust et al., 1984), decrease moss on golf course putting greens (Burnell et al., 2004), and to control the turfgrass diseases dollar spot (*Clarireedia spp*.) (McCall et al., 2017) and Microdochium patch (Mattox et al., 2016). There is only limited research into the mechanisms of how iron suppresses plant diseases. Direct iron toxicity has been speculated to reduce rust

(*Puccinia sp.*) in wheat (Forsyth, 1957) and has been shown to suppress the growth of *Clarireedia spp. in vitro* (McCall et al., 2017). A change in leaf succulence caused by osmotic effects resulting from the iron treatments has also been proposed (Cole, 1930). This osmotic effect may lead to "hardening" (Watson, 2008) of the leaves that render the turfgrass leaves less conducive to infection. Previous research into suppression of Microdochium patch on annual bluegrass putting greens by iron sulfate heptahydrate showed that the most effective rates for disease suppression (97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} applied every two weeks in 814 L ha^{-1} carrier volume) led to unacceptable turfgrass quality (Mattox et al., 2016). Golf course superintendents accustomed to applying rates similar to 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} report using carrier volumes as high as 4075 L ha^{-1} and at frequencies greater than every two weeks (Han et al., 2017). No research into the effects that these carrier volumes or frequencies have on Microdochium patch suppression have been documented.

Objectives of the research presented in chapter one of this dissertation was to gather information about how reducing the rates of mineral oil, sulfur, and phosphorous acid would affect the suppression of Microdochium patch and turfgrass quality. Chapter two focuses on answering the objective of how eliminating mineral oil applications in the winter months and how increasing application timing from two to three weeks would affect Microdochium patch suppression and turfgrass quality. Chapter three focuses on comparing application timings of iron sulfate heptahydrate on Microdochium patch suppression and turfgrass quality. Chapter four compares iron sulfate heptahydrate applied using different water carrier volumes on Microdochium patch suppression and turfgrass quality. Chapter five compares different rates of iron sulfate heptahydrate applied in combination with phosphorous acid on the suppression of Microdochium patch and turfgrass quality. During the course of this research, it was anecdotally

observed that both iron sulfate heptahydrate and phosphorous acid reduced the pH of the water carrier suspensions to as low as pH 2.2. Because previous studies found that *M. nivale* does not grow *in vitro* at a pH below 2.5 and that growth of *M. nivale* is reduced below a pH of 5.5 (Bennett, 1933), two additional experiments took place in a controlled environment (using a growth chamber) to explore if and for how long the spray suspensions may be affecting the pH of the leaf surface. In addition, a growth chamber experiment explored the effects of an acidifying agent on the suppression of Microdochium patch on annual bluegrass. Based on the results of the growth chamber experiments, additional *in vitro* experiments took place focusing on elucidating the effects of iron sulfate heptahydrate and pH on *M. nivale* in pure culture.

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Chapter 1. Comparing rates of mineral oil, sulfur, and phosphorous acid on Microdochium patch suppression, green cover percentage, and turfgrass quality

Abstract

Microdochium patch is a turfgrass disease caused by the fungal pathogen *Microdochium nivale* (Fries) Samuels and I.C. Hallett that occurs in cool-humid climates like the Pacific Northwest in North America. Mineral oil, sulfur, and phosphorous acid have been shown to suppress Microdochium patch on annual bluegrass putting greens in Western Oregon, however rates of 19.9 kg mineral oil ha^{-1} applied every two weeks alone or in combination with 12.2 kg S ha^{-1} and / or 3.7 kg phosphorous acid ha^{-1} resulted in unacceptable turfgrass thinning. The objective of this field experiment was to evaluate Microdochium patch suppression and turfgrass quality using two rates of mineral oil (10.0 kg or 19.9 kg ha^{-1}) in combination with two rates of sulfur (6.1 or 12.2 kg ha^{-1}) or two rates of phosphorous acid (1.8 or 3.7 kg ha^{-1}) compared to a non-treated control. All eight treatment combinations suppressed Microdochium patch to less than 2% disease compared to more than 40% Microdochium patch in the non-treated control in both years of the experiment. Abiotic damage represented by a loss of green cover percentage was observed in January of both years. Mineral oil applied in combination with sulfur resulted in a larger reduction of green cover percentage compared to when mineral oil was applied with phosphorous acid regardless of the rate combination, suggesting that mineral oil and sulfur combinations should be avoided in the winter months. No treatment resulted in a turfgrass quality rating considered acceptable for golf course putting greens in every month of the experiment. These findings demonstrate that lower than previously tested rates of mineral oil, sulfur, and phosphorous acid suppress Microdochium patch and merit further research into how to mitigate the loss of turfgrass quality associated with these treatments.

Introduction

Microdochium patch is a turfgrass disease that is caused by the fungal pathogen *Microdochium nivale* (Fries) Samuels & I.C. Hallett (teleomorph = *Monographella nivalis* (Schaffnit) E Müller) that occurs in cool, humid regions such as the Pacific Northwest and Northern Europe (Vargas, 2005). In the United Kingdom, Microdochium patch is reported to be a concern on the majority of golf courses (Mann and Newell, 2005). Microdochium patch occurs most commonly in these areas when temperatures are between 8 and 17°C and humidity is ≥ 90% for 20 hours or more (Dwyer et al., 2017). Microdochium patch symptoms first appear as small orange brown patches less than 5 cm in diameter and can enlarge to patches generally less than 20 cm in diameter (Smiley et al., 2005). Microdochium patch is reported to affect a number of cool season turfgrass species and can become particularly severe on annual bluegrass (Smiley et al., 2005). Annual bluegrass is the most common turfgrass grown on golf course putting greens in the Pacific Northwest (Lyman et al., 2007) and fungicides are predominantly used to manage this disease on high value areas, such as golf course putting greens (Golembiewski and McDonald, 2011; Aamlid et al., 2015). Reports of fungicide resistance (Allan-Perkins et al., 2019) and limits to fungicide chemistries permitted in certain areas (Ministère de l'agriculture et de la péche, 2006; Christie, 2010; San Francisco, 2019) is making it more challenging for golf course superintendents to manage turfgrass diseases such as Microdochium patch and alternatives to fungicides are desired.

Potential options that may be permitted where pesticide restrictions occur are organically registered fungicides (Portland, 2020) or alternative disease control products. Some mineral oils and sulfur products are registered as organic fungicides (OMRI 2020a; 2020b) and previous studies using these products to suppress Microdochium patch have shown promising results.

Mineral oil has been shown to suppress Microdochium patch on annual bluegrass research greens in Western Oregon (Mattox et al., 2020) and on a variety of mixed turfgrass stands on putting greens in Scandinavia (Aamlid et al., 2018).

Sulfur is a fungicide that has been used for plant pest control for over 2000 years (Beckerman, 2008; Smith and Secoy, 1975). Research in Western Washington has shown that annual sulfur applications of 224 kg S ha^{-1} suppresses Microdochium patch on colonial bentgrass (*Agrostis tenuis*) putting greens (Brauen et al., 1975) and research in Western Oregon has demonstrated that 12.2 kg S ha^{-1} applied every two weeks suppresses Microdochium patch on annual bluegrass putting greens (Mattox et al., 2020). Even though some sulfur products are labeled as organic (OMRI, 2020b) and the United States Environmental Protection Agency does not consider sulfur applications to be of environmental concern (EPA, 1991; Griffith et al., 2015), there are concerns with the long-term use of sulfur regarding turfgrass management. There is evidence that sulfur applications of 146 or 293 kg of S ha^{-1} or greater lead to an increase in anthracnose severity in Western Oregon compared to no sulfur additions (McDonald et al., 2018), that black layer may develop under low redox conditions in the presence of sulfur (Berndt and Vargas, 2008), and that soil pH will decrease when sulfur is applied resulting in a need for greater lime requirements (Carrow et al., 2001).

Phosphorous acid products are often marketed as fertilizers, although these phosphonate products (Guest and Grant, 1991) are reported to have fungicidal characteristics (Vincelli and Dixon, 2005) and are considered to be a low-risk pesticide regarding environmental impact and human safety (Latin, 2011). Phosphorous acid has been shown to suppress Microdochium patch on velvet bentgrass (*Agrostis canina* L. ssp. canina) and annual bluegrass in Ireland when applied at 3.8 kg H_3PO_3 ha^{-1} (Dempsey et al., 2012) and on annual bluegrass in Western Oregon

when applied at 3.7 kg H_3PO_3 ha^{-1} (Mattox et al., 2020). Some concerns remain regarding the use of phosphorous acid to control Microdochium patch. There is evidence that phosphorous acid applications do not provide any short-term phosphorus nutrition and may be detrimental to plants deficient in phosphorus (McDonald et al., 2001; Thao and Yamakawa, 2009; Dempsey, 2016). In addition, phosphorous acid is not currently permitted to be applied on golf courses in all areas across the globe, notably in Sweden (Kemikalieinspektionen, 2020).

Even though previous research in Western Oregon has demonstrated that mineral oil, sulfur, and phosphorous acid suppress Microdochium patch on annual bluegrass putting greens, abiotic damage resulted in unacceptable turfgrass quality. When mineral oil was applied every two weeks throughout the autumn and winter months at 19.94 kg a.i. ha^{-1}, unacceptable turfgrass thinning was observed and when abiotic stress was applied to plots treated with mineral oil, the greatest amount of thinning was observed (Mattox et al., 2020). The cause of turfgrass thinning is unknown, however stomatal occlusion by the mineral oil may result in reduced gas exchange (Kreuser and Rossi, 2014) resulting in a reduction in the plants ability to perform photosynthesis. Another concern with the use of frequent sulfur applications on annual bluegrass, is an increased risk of anthracnose in the summer (McDonald et al., 2018). Phosphorous acids are not permitted in certain areas to control fungal pathogens on golf courses (Kemikalieinspektionen, 2020) and therefore research into managing Microdochium patch in the absence of phosphorous acid is also desired.

Potential negative impacts of using lower rates of fungicides would include a decrease in Microdochium patch suppression (McDonald and Kowalewski, 2017) and potentially increasing the risk of fungicide resistance (van den Bosch et al., 2011) although some fungicide resistance models suggest that lower doses may decrease the risk of fungicide resistance (Mikaberidze et

al., 2017). Instances of *M. nivale* resistance *in vitro* to fungicides or reduced efficacy of fungicides in the field have already been reported for dicarboximides (Chastagner and Vassey, 1982; Pennucci et al., 1990; Gourlie and Hsiang, 2017), quinone outside inhibitors (Walker et al., 2009) and methyl benzimidazole carbamates (Huth and Schlösser, 1980; Tanaka et al., 1983; Larson, 1983). Multiple fungicide applications are often required to suppress Microdochium patch in cool-humid regions, because the weather can be conducive to disease for many months (Smiley et al., 2005) and multiple disease outbreaks are common in these climates (Mann and Newell, 2005). These conditions expose *M. nivale* to multiple fungicide applications each season, thus increasing the risk of fungicide resistance (Latin, 2011). Even though no resistance has been reported for *M. nivale* to mineral oil, sulfur, or phosphorous acid, there is a concern that frequent applications of the same products may select for fungal strains resistant to these materials. In addition, both mineral oil and phosphorous acid are reported to induce host defense (Cortes-Barco et al., 2010b and Dempsey, 2016) and there is some speculation that if the plant's natural defense system is continuously activated, there could potentially be a risk of selection pressure of a mutational change of the plant pathogen leading to future resistance (Hewitt, 1998).

No research exists that explores the use of combinations of mineral oil rates lower than 19.9 kg a.i. ha^{-1}, sulfur rates lower than 12.2 kg ha^{-1}, or phosphorous acid rates lower than 3.7 kg ha^{-1} applied every two weeks on the suppression of Microdochium patch or on turfgrass quality from the fall through spring. The objective of this field experiment was to quantify two different rates of sulfur and phosphorous acid, in combination with two different rates of mineral oil in order to assess if differences in disease suppression or abiotic turfgrass stress would be observed.

Materials and methods

Experimental design

A field trial was conducted on an annual bluegrass putting green in Corvallis, OR at the Lewis-Brown Horticulture Farm (latitude: 44.549668, longitude: -123.216139). The putting green was built in May 2009 by placing 30 cm of United States Golf Association recommended particle size sand (USGA, 2018) on the native Malabon silty clay loan (UC Davis, 2019). Annual bluegrass sod grown on sand was used to establish the green (Bos Sod, Abbotsford, BD, Canada). The experiment took place from 21 Sep 2015 to 7 Apr 2016 and was repeated from 22 Sep 2016 to 7 Apr 2017 on a different section of the same green. Microdochium patch occurred naturally at the site. The mean temperatures during the experimental period were 1.2°C higher in the first year and -0.3°C cooler in the second year compared to the 30-year average from 1981 to 2000 (NOAA, 2020) (Table 1.1).

The study arrangement was a 2 X 4 factorial plus a non-treated control. The treatments consisted of two rates of the mineral oil and copper (Cu) II pthalocyanine green pigment (Civitas Turf Defense, HollyFrontier Specialty Products, Mississauga, Ontario) applied at 10.0 kg a.i. ha^{-1} (13.5 L ha^{-1}) or 19.9 kg a.i. ha^{-1} (27 L ha^{-1}) and four rates of fertilizer; phosphorous acid applied at 3.7 kg H_3PO_3 ha^{-1} as GRIGG P-K Plus (Brandt Consolidated, Springfield, IL) with a fertilizer formulation of 3-5-17, and elemental sulfur (Sulfur DF, Wilbur-Ellis, San Francisco, CA) applied at 6.1 kg S ha^{-1} or 12.2 kg S ha^{-1} in addition to a non-treated control. The experimental plots were 1.5 m^2 and the total experimental area was 54 m^2.

Applications occurred every two weeks with 14 applications made in the first year. Because of weather delays, only 13 applications were made in the second year. A handheld boom with four XR80015 nozzles (TeeJet Technologies, Independence, OR) attached to a CO_2

pressurized backpack sprayer with a boom pressure of 280 kPa was used to apply the treatments. Spray carrier volume was 1019 L ha^{-1} and a metronome was used to ensure a consistent walking speed. Treatments were left on the foliage to dry.

Turfgrass maintenance

Throughout the trial period spanning from late September to early April, the green was mown at 3.8 mm once a week with clippings removed. Foot traffic replication representing 73 golf rounds a day (average winter play at a local municipal course in Corvallis, OR) took place throughout the trial by walking over the plots with golf shoes following the protocol described by Hathaway and Nikolai (2005). In both years, urea was added to each spray treatment to account for the nitrogen applied with the phosphorous acid product for a total nitrogen input of 4.9 kg N ha^{-1} every two weeks for a total applied nitrogen of 83.3 kg N ha^{-1} during each year of the study periods. Because of the precipitation throughout the trial (Table 1.1), no irrigation was applied during the trial period. In the summer between trial years, irrigation was applied to replace 80% of the daily evapotranspiration. In year one, cool season brown patch (*Ceratobasidium cereale* D. Murray & L.L. Burpee) and dollar spot (*Clarireedia* spp.) appeared on the trial, therefore flutolanil [N-[3-(1-methylethoxy)phenyl]-2-(trifluoromethyl)benzamide] was applied on 29 Sep 2015 and boscalid [3-pyridinecarboxamide, 2-chloro-N-(4´-chloro(1,1´-biphenyl)-2-yl)] was applied on 19 Oct 2015. Research suggests that Microdochium patch is not effectively suppressed by either flutolanil (Settle et al., 2011) or boscalid (Popko et al., 2016). No other fungicides were applied during the study period. In the summer between trial years, applications of fungicides were applied on a preventative basis against anthracnose (*Colletotrichum cereale* Manns). In order to suppress creeping bentgrass on the green,

sethoxydim [2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one] was applied in the summer between trial years.

Response variables

The response variables included; disease percentage, area under disease progress curve (AUDPC), abiotic damage quantified as green cover percentage, and turfgrass quality. In order to calculate disease percentage and green cover percentage, two digital images per plot were collected monthly using a Sony DSC-H9 camera mounted onto a 0.31 m^2 light box with four 40-W spring lamps (TPC, Lighthouse supply, Bristol, VA) powered by a battery-pack. Because of the presence of turfgrass thinning and the accompanying loss of green cover percentage, digital image analysis was rendered impractical for assessing disease. For this reason, disease percentage was calculated using stratified sampling (Laycock and Canaway, 1980; Richardson et al., 2001) by overlaying a digital 100-point grid onto each image using Sigma Scan Pro version 5.0 (Systat Software, Inc., San Jose, CA). These disease percentage data were used to calculate AUDPCs from the beginning of the trial up to the peak of disease for each year following the trapezoidal method (Shaner and Finney, 1977). To quantify abiotic damage resulting from the treatments, digital image analysis was used to calculate green cover percentage by analyzing the digital images of the eight treatments of mineral oil and sulfur or mineral oil and phosphorous acid (the non-treated control was not included in the analysis because no abiotic damage was observed) using the Turf Analyzer program (Green Research Services, Fayetteville, Arkansas) (Karcher and Richardson, 2013) with the following settings: hue: 75 to 360, saturation: 45 to 100, and brightness: 0 to 74. The National Turfgrass Evaluation Program turfgrass quality rating

system was used to assess turfgrass quality using a rating from 1 to 9 with a rating of 6 or greater considered acceptable for golf course putting greens (Krans and Morris, 2007).

Statistical analyses

Because many of the treatments completely suppressed Microdochium patch and because there was little and sometimes the complete absence of variance in the disease percentage and AUDPC data, further statistical analysis options were limited. For this reason, a permutation test was performed in R (R Core Team, 2018) using one million simulations of the disease percentage and AUDPC data individually for each year in this experiment to test the probability that the results observed in each year could have been because of random chance (Ramsey and Shafer, 2002). Monthly green cover percentage data were analyzed in a repeated measures analysis of variance, standard t-tests were used to make multiple comparisons, and family-wise errors were corrected using the Holm's method (Holm, 1979) using Proc Mixed in SAS 9.4 (SAS Institute Inc., Cary, N.C). The ordinal turfgrass quality data (Karcher, 2000) was analyzed using the Kruskal-Wallis (Kruskal and Wallis, 1952) and pairwise comparisons were assessed using Dunn's test (Dunn, 1964) in R.

Results

Disease percentage and AUDPC

Disease began to appear in November (10 November 2015 and 3 November 2016) of each year with an average Microdochium patch percentage above 40% in the non-treated control at the peak of disease in both years of the experiment (Figures 1.1 and 1.2). No Microdochium patch was observed in either year on plots that received 19.9 kg of mineral oil ha^{-1} in combination with either 6.1 kg S ha^{-1}, 1.8 kg H_3PO_3 ha^{-1}, or 3.7 kg H_3PO_3 ha^{-1}. Some

Microdochium patch was observed on the other treatments during at least one year of this two-year study, however Microdochium patch was always less than 2% at the peak of disease for all treatments. The permutation tests resulted in a $P < 0.001$ for the peak of disease in both years and $P < 0.001$ for the AUDPC in both years. These tests provide strong evidence that the results observed were not because of random chance (i.e. the suppression of Microdochium patch observed was a result of the experimental treatments).

Green cover percentage

The repeated measures analysis of green cover percentage indicated a significant interaction between mineral oil and month ($P<0.001$) and between fertility and month ($P<0.001$) in both years. The average green cover percentage was above 95% for all treatments in November in both years, decreased in December and January for certain treatments and had returned to 94% or greater by February (Tables 1.2 and 1.3; Figure 1.3). In both years, the loss of green cover percentage on treatments receiving mineral oil and sulfur was the most striking in January, while the mineral oil and phosphorous acid combination had the highest green cover percentage in January (> 96%). The lowest green cover percentage in January was observed when 10.0 or 19.9 kg mineral oil ha^{-1} was applied with 12.2 kg S ha^{-1} in year one and when 19.9 kg mineral oil ha^{-1} was applied with 12.2 kg S ha^{-1} in year two.

Turfgrass quality

Turfgrass quality data were inconsistent over the two years with no treatment receiving an acceptable turfgrass quality of 6 or greater in every month in both years. In year one, on the January and February rating date, the combination of 19.9 kg mineral oil ha^{-1} in combination

with 12.2 kg S ha^{-1} resulted in the lowest turfgrass quality rating because of abiotic damage expressed as a reduction in green cover percentage. In March and April in the first year and every month in the second year, the non-treated control resulted in the lowest turfgrass quality because of a higher percentage of Microdochium patch severity (Tables 1.4 and 1.5). The rate of 19.9 kg mineral oil applied in combination with either 1.8 or 3.7 kg phosphorous acid ha^{-1} resulted in acceptable turfgrass quality in every month with the exception of one month in one of the two years. In those instances, the turfgrass color was not considered acceptable because of abiotic damage from the treatments.

Discussion

This current experiment has demonstrated that rates as low as 13.5 L (10.0 kg) mineral oil ha^{-1} in combination with either 1.8 or 3.7 kg phosphorous acid ha^{-1} every two weeks suppressed Microdochium patch by 99.3% and 99.7% in year one and 96.5% and 99.8% in year two respectively. This provides evidence that two-week application intervals of lower than previously tested combination rates of mineral oil with sulfur or phosphorous acid suppress Microdochium patch throughout the fall and winter in Western Oregon.

Previous studies have demonstrated that every 2-week applications of 19.9 kg of mineral oil ha^{-1} applied either alone or in combination with 3.7 kg phosphorous acid ha^{-1} and/or with 12.2 kg sulfur ha^{-1} suppressed Microdochium patch compared to a non-treated control (Mattox et al., 2020). Other research in Scandinavia exploring the use of 27 or 54 L ha^{-1} of mineral oil in combination with 3 kg phosphorous acid ha^{-1} every three weeks resulted in a 94% and 98% Microdochium patch suppression respectively compared to a non-treated control (Aamlid, 2018). While the lower rates used in this current experiment applied at a two-week frequency may

increase the labor costs compared to a three-week application frequency, they will result in a decrease in the environmental impact compared to higher rates (Kovach et al., 1992) and reduce pesticide exposure to pesticide handlers (Environmental Protection Agency, 2020).

Relying solely on the use of mineral oil, sulfur, and phosphorous acid for multiple months may select for conditions favorable for fungicide resistance and caution should be used when building a fungicide program based on frequent applications of only these three products. Fungicide research suggests that there is an increased risk of fungicide resistance when repeated fungicide applications of the same chemistries are used (van den Bosch et al., 2014), especially for systemic fungicides that are single-site inhibitors (Hewitt, 1998). The mode of action of phosphorous acid is considered to induce host plant resistance (Fungicide Resistance Action Committee, 2019). There is also evidence that phosphorous acid applications reduce *M. nivale* conidial germination and cause morphological deformities in hyphae suggesting a fungistatic effect (Dempsey et al., 2012). Despite this multi-pronged evidence of activity, reduced sensitivity has already been reported for the lettuce downy mildew pathogen (*Bremia lactucae*) to the phosphonate Aliette [Aluminum tris (O-ethyl phosphonate)] (Bayer AG, Leverkusen, Germany) when it was repeatedly applied to lettuce fields in California (Brown et al., 2004). Even though *B. lactucae* is not a fungus (Fletcher et al., 2019), the fact that plant disease resistance to phosphonates has occurred suggests that repeated applications of phosphorous acid on turfgrass could potentially lead to reduced sensitivity (Vincelli, 2004). The mode of action of mineral oils are not classified with an unknown target site and no known resistance (Fungicide Resistance Action Committee, 2019). The mineral oil tested in this study has previously been shown to induce systemic resistance in creeping bentgrass (*Agrostis stolonifera*) (Cortes-Barco et al., 2010a) where suppression of Microdochium patch was observed when the mineral oil was

applied to the soil suggesting that mineral oil may be affecting the plant via root uptake or affecting the soil microorganisms (Cortes-Barco et al., 2010b). Some transient evidence of fungistatic activity was also reported for mineral oil against *M. nivale in vitro* (Cortes-Barco et al., 2010a), however if the principal activity of mineral oils is related to plant defense and does not primarily affect the pathogen, the risk of fungicide resistance is considered to be low. Sulfur is considered to have multi-site activity with a low risk of resistance (Hewitt, 1998; Fungicide Resistance Action Committee, 2019) and no reports of resistance to sulfur applications is readily available in the literature in spite of its use as a fungicide for over 2000 years (Smith and Secoy, 1975).

A reduction in the amount of mineral oil applied from 19.9 to 10.0 liters ha^{-1} did not improve the abiotic damage observed quantified as green cover percentage in either year, however the reduction in sulfur from 12.2 to 6.1 kg ha^{-1} did result in a greater green cover percentage compared to the highest mineral oil and sulfur combinations. The reason for the loss of green cover is unclear, however, a reduction in photosynthesis has been reported in grapes when oil was applied followed by a sulfur application (Baudoin et al., 2006) and in apple trees when oil and sulfur were applied at the same time (Ferree et al., 1999). The loss of photosynthesis in grape leaves by oil applications is speculated to be caused by a decrease in gas exchange (Finger et al., 2002) and stomatal occlusion of turfgrass leaves by mineral oil applications has also been associated with a decrease in gas exchange (Kreuser and Rossi, 2014). Photosynthesis was shown to reduce in summer when pigments were applied to creeping bentgrass (McCarty et al., 2014) and microscopy indicates that application of some pigments may also lead to stomatal occlusion (McCarty et al., 2013). No published literature concerning effects of sulfur applications on photosynthesis exist for turfgrass, although sulfur applications as

micronized wettable sulfur and magnetic spray wettable sulfur have been shown to reduce photosynthesis in apple leaves. The mechanisms behind the reduction in photosynthesis were not clear, although some reduction in light intensity striking the leaves caused by the sulfur applications was speculated (Hyre, 1939). It could be hypothesized that any reduction of photosynthesis when day-lengths are shortened would reduce the capacity of annual bluegrass plants to recuperate from abiotic stresses. If the loss of green cover percentage in this current experiment is a form of abiotic stress caused by a reduction in photosynthesis, that could explain why this stress was manifested the most in plots receiving the highest rates of mineral oil and sulfur.

Another possible mechanism behind the loss of green cover percentage may come from a carbon drain caused by a continuous priming of plant defenses (Vos et al., 2013). The loss of green cover percentage in this study was greatest when mineral oil was applied in combination with sulfur. The mineral oil used in this research has been shown to lead to an expression of genes associated with jasmonic acid synthesis in creeping bentgrass (Cortes-Barco et al., 2010a) which can lead to induced systemic resistance in plants (Vallad and Goodman, 2004), possibly allocating carbon resources to plant defense at the detriment of growth (Heil and Baldwin, 2002). These negative effects may increase in the colder months of the winter when daylengths are still short and temperatures are lower than more favorable growth conditions (Vargas and Turgeon, 2004). If sulfur applications are applied in combination with mineral oil, a potential decrease in photosynthesis may lead to even more abiotic damage (further reduction in green cover percentage) (Hyre, 1939).

Temperature may also play a role in the loss of green cover percentage observed. Somewhat surprisingly, the reduction in green cover percentage in the mineral oil and sulfur

treated plots appeared to be more pronounced in the first year compared to the second year (Table 1.2 and 1.3), even though the weather was cooler in the second year compared to the first year (Table 1.1). Photosynthetic processes have been observed to take place in grasses at temperatures as low as - 4°C (Skinner, 2007; Höglind et al., 2011), with the minimum temperature for photosynthesis considered to be when water in the leaf tissues is not frozen (Pisek, 1973). There was at least one day in November, December, January, and February in the first year where the minimum temperature was below freezing, whereas the minimum temperature in November never dipped below freezing in the second year (Table 1.1). It could be speculated that the early freezing events caused a reduction in photosynthetic activity and growth of annual bluegrass in November of the first year causing the difference observed between the two years.

Concerning turfgrass quality, a decrease in ratings was observed because of the presence of Microdochium patch, the presence of abiotic damage quantified by a reduction in green cover percentage, and/or a decrease in turfgrass color. The applications of 19.9 kg mineral oil in combination with 1.8 or 3.7 kg phosphorous acid ha^{-1} every two weeks were considered acceptable in all months except for one month in each year. Considering the increased pesticide restrictions in certain areas and reports of *M. nivale* resistance to certain fungicides, there may be a shift in the future as to what would be considered acceptable turfgrass quality in the winter months if disease-free turfgrass can be assured for the spring. Where fungicides are available that do not result in unacceptable turfgrass quality in the winter season, it is likely that golfers and turfgrass managers will not accept a reduction in putting green performance (Walsh, 2005). In areas where no fungicides are available, perhaps stakeholders in those areas will tolerate a

decrease in turfgrass quality ratings compared to over 40% Microdochium patch as was observed in the non-treated control plots in this current experiment.

Future studies are warranted to look into the long-term effects of these applications on their effects on soil pH, the incidence of these fall and winter applications on summer diseases, and possible effects on fertility (such as copper from the pigmented mineral oil and phosphorous from the phosphorous acid). In this trial, 4.9 kg N ha^{-1} was applied every two weeks and no plant growth regulators were used. Future research is needed to determine if turfgrass thinning would be affected if a different fertility program or plant growth regulator program were used in combination with these applications.

Conclusion

This field experiment demonstrated that lower than previously tested rates of mineral oil, sulfur, and phosphorous acid suppress Microdochium patch throughout the fall and winter in Western Oregon. Strong evidence was also provided showing that the combination of mineral oil and sulfur resulted in a greater loss of green cover percentage compared to a mineral oil and phosphorous acid combination, suggesting that mineral oils and sulfur should not be applied in combination on turfgrass in the winter months. Future research is warranted that focuses on the exclusion of mineral oils in the winter months as well as long term impacts of applications of mineral oil, sulfur, and phosphorous acid specifically regarding summer turfgrass pathogens such as anthracnose, changes in soil pH, and long term impacts on soil nutrition such as phosphorous and copper levels in the soil.

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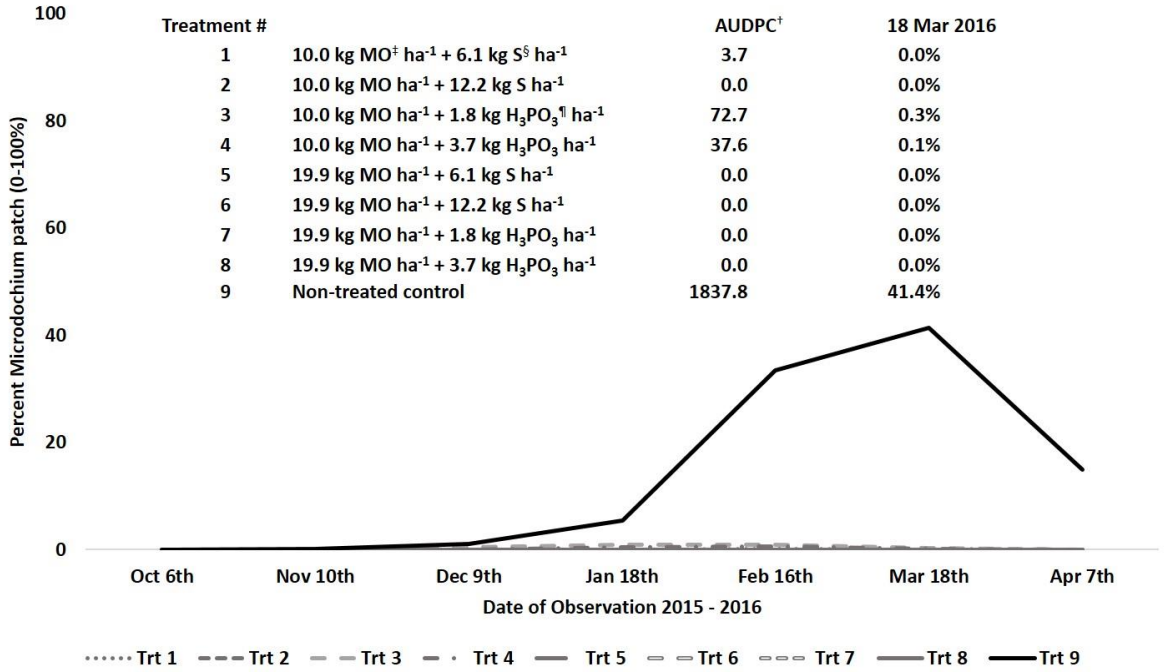

Figure 1.1 Effects of treatments on area under disease progress curve and disease percentage on an annual bluegrass putting green in Corvallis, OR. † AUDPC = Area under disease progress curve calculated from the trial start date on 21 Sep 2015 up to the peak of disease on 18 Mar 2016. ‡ MO = Civitas Turf Defense applied every two weeks. § S = Sulfur applied every two weeks. ¶ H_3PO_3 = Phosphorous acid applied every two weeks.

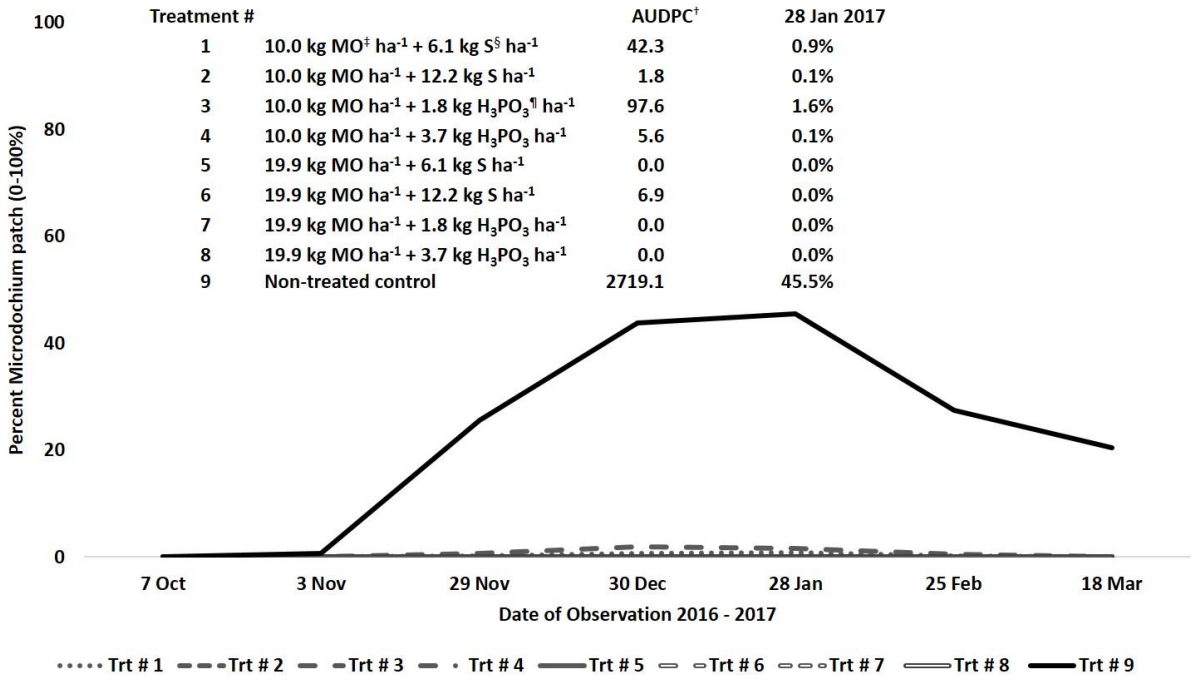

Figure 1.2 Effects of treatments on area under disease progress curve and disease percentage on an annual bluegrass putting green in Corvallis, OR. † AUDPC = Area under disease progress curve calculated from the trial start date on 22 Sep 2016 up to the peak of disease on 28 Jan 2017. ‡ MO = Civitas Turf Defense applied every two weeks. § S = Sulfur applied every two weeks. ¶ H_3PO_3 = Phosphorous acid applied every two weeks.

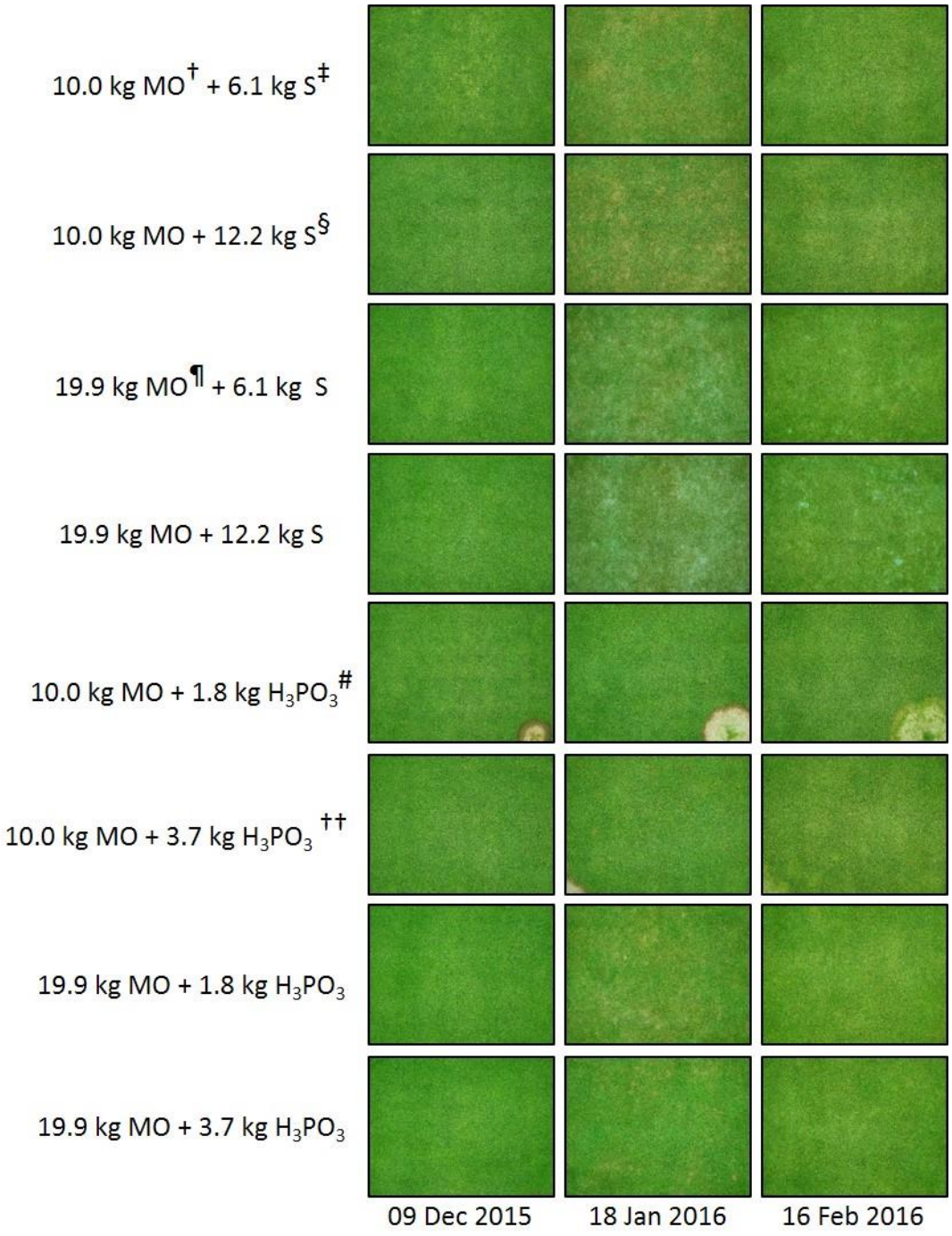

Figure 1.3. Images of representative plots from 09 Dec 2015 to 16 Feb 2016. †10.0 kg Civitas Turf Defense ha^{-1} applied every two weeks. ‡ 6.1 kg S ha^{-1} applied every two weeks. § 12.2. kg S ha^{-1} applied every two weeks. ¶ 19.9 kg Civitas Turf Defense ha^{-1} applied every two weeks. # 1.8 kg H_3PO_3 ha^{-1} applied every two weeks. †† 3.7 kg H_3PO_3 ha^{-1} applied every two weeks.

Corvallis, OR		2015-2016 Lewis-Brown Research Farm				2016-2017 Lewis-Brown Research Farm			
30 year mean temp 1981- 2010†		Mean temp	+/- 30 yr mean	Min temp	Max temp	Mean temp	+/- 30 yr mean	Min temp	Max temp
September	16.8°C	15.8	-1.0	3.6	32.6	15.9	-1.0	3.6	31.1
October	11.8	14.0	2.3	2.9	27.2	12.2	0.5	2.3	22.5
November	7.3	6.5	-0.9	-6.6	17.3	9.9	2.5	2.5	20.3
December	4.2	6.3	2.1	-3.1	16.1	2.6	-1.6	-6.8	11.9
January	4.8	5.4	0.6	-5.7	16.7	1.8	-3.0	-9.6	12.0
February	6.0	8.1	2.1	-0.5	16.7	5.5	-0.5	-1.5	15.6
March	8.2	9.0	0.8	0.6	21.5	8.4	0.3	0.2	18.2
April	10.1	12.3	2.2	0.9	29.8	9.5	-0.6	0.1	19.5
Averages:	8.5	9.7	1.2	-1.0	22.2	8.2	-0.3	-1.1	18.9

Table 1.1. Weather data summary for the Lewis-Brown Turfgrass Research Farm in Corvallis, OR from September 2015 to April 2016 and from September 2016 to April 2017. †Thirty-year average monthly data collected on 04 February 2020 from the National Oceanic and Atmospheric Administration (https://www.ncdc.noaa.gov/cdo-web/datatools/normals).

	2015 - 2016[†]			
	10-Nov	09-Dec	18-Jan	16-Feb
10.0 kg MO[‡] ha^{-1} + 6.1 kg S[§] ha^{-1}	94.9[¶] a[#]	97.4 a	90.4 c	95.1 bc
10.0 kg MO ha^{-1} + 12.2 kg S ha^{-1}	95.9 a	97.1 a	79.0 e	93.8 c
10.0 kg MO ha^{-1} + 1.8 kg H_3PO_3[††] ha^{-1}	97.1 a	97.6 a	96.3 ab	94.6 c
10.0 kg MO ha^{-1} + 3.7 kg H_3PO_3 ha^{-1}	96.2 a	97.5 a	96.5 a	94.1 c
19.9 kg MO ha^{-1} + 6.1 kg S ha^{-1}	96.9 a	97.6 a	91.7 bc	97.9 a
19.9 kg MO ha^{-1} + 12.2 kg S ha^{-1}	96.7 a	97.3 a	82.6 e	97.4 a
19.9 kg MO ha^{-1} + 1.8 kg H_3PO_3 ha^{-1}	97.9 a	98.1 a	96.9 a	97.0 ab
19.9 kg MO ha^{-1} + 3.7 kg H_3PO_3 ha^{-1}	97.4 a	98.0 a	97.0 a	97.0 ab

Table 1.2 Effects of treatments on green cover percentage on an annual bluegrass research green in Corvallis, OR. [†] Trial began on 21 Sep 2015 and concluded on 7 Apr 2016. [‡] MO = Civitas Turf Defense ha^{-1} applied every two weeks. [§] S = Sulfur applied every two weeks. [¶] Columns within each month represent the mean green cover percentage. [#] The letters in each column indicate significant differences between the treatments after using standard t-tests and controlling for multiple comparisons within each month using Holm's method (alpha≤0.05). [††] H_3PO_3 = Phosphorous acid applied every two weeks.

	2016 - 2017							
	29-Nov		30-Dec		28-Jan		25-Feb	
10.0 kg MO[§] ha^{-1} + 6.1 kg S[¶] ha^{-1}	95.9	ab	96.0	ab	96.5	b	98.2	b
10.0 kg MO ha^{-1} + 12.2 kg S ha^{-1}	96.3	ab	94.8	ab	94.7	bc	97.9	ab
10.0 kg MO ha^{-1} + 1.8 kg H_3PO_3[††] ha^{-1}	96.2	ab	96.7	a	98.0	a	98.6	ab
10.0 kg MO ha^{-1} + 3.7 kg H_3PO_3 ha^{-1}	95.9	ab	97.4	a	98.4	a	98.4	ab
19.9 kg MO ha^{-1} + 6.1 kg S ha^{-1}	97.2	ab	94.8	b	94.3	c	98.0	b
19.9 kg MO ha^{-1} + 12.2 kg S ha^{-1}	95.3	b	90.1	c	88.0	d	93.8	ab
19.9 kg MO ha^{-1} + 1.8 kg H_3PO_3 ha^{-1}	97.7	ab	97.0	a	97.9	a	98.9	ab
19.9 kg MO ha^{-1} + 3.7 kg H_3PO_3 ha^{-1}	97.9	a	97.1	a	98.0	a	98.9	a

Table 1.3 Effects of treatments on green cover percentage on an annual bluegrass research green in Corvallis, OR. [†] Trial began on 22 Sep 2016 and concluded on 7 Apr 2017. [‡] MO = Civitas Turf Defense ha^{-1} applied every two weeks. [§] S = Sulfur applied every two weeks. [¶] Columns within each month represent the mean green cover percentage. [#] The letters in each column indicate significant differences between the treatments after using standard t-tests and controlling for multiple comparisons within each month using Holm's method (alpha≤0.05). [††] H_3PO_3 = Phosphorous acid applied every two weeks.

		Year One - 2015 to 2016[†]					
		- Pr > F -					
Source of Variation	DF	13-Nov	16-Dec	25-Jan	16-Feb	18-Mar	01-Apr
Treatment	8	P=.003[‡]	P=.078	P=.003	P<.001	P<.001	P<.001
		13-Nov	16-Dec	25-Jan	16-Feb	18-Mar	01-Apr
10.0 kg MO[§] ha^{-1} + 6.1 kg S[¶] ha^{-1}		6.9[#] a[††]	6.5 a	5.9 ab	6.6 ab	7.8 a	7.5 a
10.0 kg MO ha^{-1} + 12.2 kg S ha^{-1}		7.4 a	7.4 a	5.5 ab	7.0 a	6.0 ab	6.0 ab
10.0 kg MO ha^{-1} + 1.8 kg H_3PO_3[††] ha^{-1}		7.1 a	6.5 a	6.5 a	6.3 ab	7.0 ab	6.9 ab
10.0 kg MO ha^{-1} + 3.7 kg H_3PO_3 ha^{-1}		6.3 a	6.3 a	6.1 ab	5.9 ab	6.3 ab	6.4 ab
19.9 kg MO ha^{-1} + 6.1 kg S ha^{-1}		7.5 a	6.3 a	4.5 ab	4.0 ab	8.0 a	7.0 ab
19.9 kg MO ha^{-1} + 12.2 kg S ha^{-1}		7.5 a	6.4 a	3.3 b	3.0 b	5.1 ab	5.5 ab
19.9 kg MO ha^{-1} + 1.8 kg H_3PO_3 ha^{-1}		7.5 a	7.5 a	5.8 ab	6.8 ab	7.8 a	7.3 ab
19.9 kg MO ha^{-1} + 3.7 kg H_3PO_3 ha^{-1}		7.5 a	7.4 a	6.3 a	6.8 ab	8.0 a	7.5 a
Non-treated control		6.3 a	5.3 a	5.0 ab	4.0 ab	3.0 b	3.3 b

Table 1.4. Kruskal-wallis nonparametric one-way analysis of variance and effects of treatments on turfgrass quality ratings on an annual bluegrass research green in Corvallis, OR. [†] Trial began on 21 Sep 2015 and concluded on 7 Apr 2016. [‡] Corrections within the month were applied using the Holm's method within each month. [§] MO = Civitas Turf Defense ha^{-1} applied every two weeks. [¶] S = Sulfur applied every two weeks. [#] Columns within each month represent the mean turfgrass quality rating. [††] The letters in each column indicate significant differences between the treatments after using Dunn's test and controlling for multiple comparisons within each month using Holm's method (alpha≤0.05).

		Year Two - 2016 to 2017[†]					
		- Pr > F -					
Source of Variation	DF	18-Nov	23-Dec	29-Jan	25-Feb	16-Mar	16-Apr
Treatment	8	P = .003[‡]	P = .007	P < .001	P < .001	P = .002	P < .001
Treatment		18-Nov	23-Dec	29-Jan	25-Feb	16-Mar	16-Apr
10.0 kg MO[§] ha^{-1} + 6.1 kg S[¶] ha^{-1}		6.1[#] ab[††]	5.4 ab	5.3 ab	5.6 ab	6.3 ab	6.8 ab
10.0 kg MO ha^{-1} + 12.2 kg S ha^{-1}		6.6 ab	6.1 a	6.1 a	6.5 a	6.5 ab	6.0 ab
10.0 kg MO ha^{-1} + 1.8 kg H_3PO_3[‡‡] ha^{-1}		6.0 ab	5.0 ab	5.1 ab	5.4 ab	5.8 ab	7.0 a
10.0 kg MO ha^{-1} + 3.7 kg H_3PO_3 ha^{-1}		7.0 a	5.8 ab	6.3 a	6.8 a	6.5 ab	7.0 a
19.9 kg MO ha^{-1} + 6.1 kg S ha^{-1}		7.0 a	5.3 ab	4.3 ab	5.0 ab	6.5 ab	7.0 a
19.9 kg MO ha^{-1} + 12.2 kg S ha^{-1}		6.1 ab	5.0 ab	3.0 ab	4.0 ab	4.0 ab	5.0 ab
19.9 kg MO ha^{-1} + 1.8 kg H_3PO_3 ha^{-1}		6.5 ab	6.1 a	6.1 a	6.8 a	7.0 a	7.0 a
19.9 kg MO ha^{-1} + 3.7 kg H_3PO_3 ha^{-1}		7.0 a	5.8 ab	6.0 ab	6.8 a	6.8 a	7.0 a
Non-treated control		4.0 b	3.0 b	1.8 b	2.8 b	2.5 b	4.3 b

Table 1.5. Kruskal-wallis nonparametric one-way analysis of variance and effects of treatments on turfgrass quality ratings on an annual bluegrass research green in Corvallis, OR. [†] Trial began on 22 Sep 2016 and concluded on 7 Apr 2017. [‡] Corrections within the month were applied using the Holm's method within each month. [§] MO = Civitas Turf Defense applied every two weeks. [¶] S = Sulfur applied every two weeks. [#] Columns within each month represent the mean turfgrass quality rating. [††] The letters in each column indicate significant differences between the treatments after using Dunn's test and controlling for multiple comparisons within each month using Holm's method (alpha≤0.05). [‡‡] H_3PO_3 = Phosphorous acid applied every two weeks.

Chapter 2. Suppression of Microdochium patch using rotations of mineral oil, sulfur, and phosphorous acid

Abstract

Microdochium patch is a turfgrass disease caused by the fungal pathogen *Microdochium nivale* (Fries) Samuels and I.C. Hallett that occurs in cool-humid conditions, which are commonplace in the United States Pacific Northwest and Northern Europe. Pesticide restrictions in certain areas are making it a challenge to suppress Microdochium patch and alternative methods of disease control are desired. Previous research has shown that mineral oil, sulfur, and phosphorous acid have the potential to suppress Microdochium patch, however abiotic damage was associated with repeated mineral oil applications. Two field experiments taking place in Western Oregon are presented in this manuscript. The objective of the first field experiment was to compare applications consisting of combinations of mineral oil and sulfur or mineral oil and phosphorous acid in rotation with combinations of sulfur and phosphorous acid regarding the suppression of Microdochium patch and turfgrass quality. The objective of the second field experiment focused on seasonal rotations that excluded mineral oil in certain months and compared two- and three-week application frequencies. All the rotations, in both experiments, suppressed Microdochium patch compared to the non-treated control, although differences in turfgrass quality were observed. In the first experiment, the mineral oil and phosphorous acid combination rotated with sulfur and phosphorous acid suppressed Microdochium patch and resulted in the highest green cover percentage. At the peak of disease in the second experiment, no Microdochium patch was observed when applications were applied every two weeks, except for the sulfur and phosphorous acid combination applied every two weeks. Two treatments applied every two weeks that withheld mineral oil in the winter months resulted in turfgrass quality considered acceptable for golf course putting greens on all rating dates.

Introduction

Microdochium patch is a turfgrass disease caused by the fungal pathogen *Microdochium nivale* (Fries) Samuels & I.C. Hallett (teleomorph = *Monographella nivalis*). This disease predominantly occurs in cool-humid areas when temperatures are between 8 and 17°C and when the relative humidity is greater than 90% for 20 hours or more (Dwyer et al., 2017). Microdochium patch commonly occurs in areas like the Pacific Northwest (Smiley et al., 2005; Vargas, 2005) and in the United Kingdom (Mann and Newell, 2005) where freezing conditions and snow cover is rare. Disease symptoms appear as small patches, orange brown in color less than 5 cm in diameter with patches usually not enlarging greater than 20 cm in diameter (Smiley et al., 2005). Annual bluegrass is the most common turfgrass managed on golf course putting greens in the Pacific Northwest (Lymann et al., 2007) and even though most cool-season grasses are susceptible to Microdochium patch, annual bluegrass is particularly prone to this disease (Smiley et al., 2005). There are multiple fungicides that are effective at suppressing Microdochium patch (Golembiewski and McDonald, 2011; Aamlid et al., 2015), however pesticide restrictions in some parts of the world (Ministère de l'agriculture et de la pêche, 2006; Christie, 2010; San Francisco, 2020) and the risk of fungicide resistance (Allan-Perkins et al., 2019) are encouraging turfgrass managers to seek alternatives to traditional fungicides.

Alternatives to traditional fungicides that have been shown to suppress Microdochium patch include mineral oil on creeping bentgrass (Cortes-Barco et al., 2010b), sulfur on colonial bentgrass (Brauen et al., 1975), and phosphorous acid on annual bluegrass and velvet bentgrass (Dempsey et al., 2012). Some mineral oils (OMRI, 2020a) and sulfur products (OMRI, 2020b) are registered for organic agriculture and phosphorous acid is available as a fertilizer. These

types of products may be permitted in areas where other chemicals are not permitted (Portland, 2020). Even though some phosphorous acid containing products are registered as fertilizers, phosphorous acid is considered to be a low-risk pesticide regarding environmental impact and human safety (Latin, 2011) with some phosphorous acid products registered as fungicides. There are no known reports of *M. nivale* resistance to mineral oils, sulfur, or phosphorous acid. Loss of turfgrass density from mineral oil applications has been reported (Kreuser and Rossi, 2014; Mattox et al., 2020), with a decrease in green cover percentage being exacerbated when mineral oils are combined with sulfur during the winter months (Chapter 1 of this dissertation). Sulfur applications have also been linked to an increase in summer anthracnose severity (McDonald et al., 2018), therefore reducing total applied sulfur in the fall and winter would be desirable to mitigate adverse effects on anthracnose.

In research focusing on using lower rates of different combinations of mineral oil with sulfur or phosphorous acid, a reduction in green cover percentage was caused by abiotic damage resulting from the treatments. The greatest loss in green cover percentage was observed whenever sulfur was applied in combination with mineral oils, regardless of the rate of sulfur, compared to treatments not receiving sulfur (Chapter 1 of this dissertation). Other research demonstrated that a combination of sulfur and phosphorous acid did not result in abiotic damage and that this combination suppressed Microdochium patch more than when sulfur or phosphorous acid were applied alone, although some disease did occur (Mattox et al., 2020). More research is therefore needed regarding the use of mineral oil, sulfur, and phosphorous acid throughout the fall and winter to suppress Microdochium patch to levels comparable to traditional fungicides and to maintain acceptable turfgrass quality throughout the fall and winter.

The only published research to date focusing on using combinations of mineral oil with phosphorous acid to suppress Microdochium patch in the absence of snow cover took place in Scandinavia (Aamlid et al., 2018) and Western Oregon (Mattox et al., 2020), therefore only limited information is available regarding the efficacy of different application intervals and no study, thus far, has focused on seasonal rotations of these product combinations. In the Scandinavian study, 27 and 54 L mineral oil ha^{-1} was applied in combination with 3 kg phosphorous acid ha^{-1} every three weeks suppressing Microdochium patch by 94% and 98% respectively (Aamlid et al., 2018). In Western Oregon, 19.9 kg (27 L) mineral oil ha^{-1} in combination with 3.7 kg phosphorous acid ha^{-1} suppressed Microdochium patch although unacceptable turfgrass thinning occurred (Mattox et al., 2020).

This current manuscript focuses on two different field experiments. The primary objective of the first experiment was to compare rotations that included mineral oil in combination with sulfur or phosphorous acid rotated with a combination of sulfur and phosphorous acid. The hypothesis of this experiment was that replacing every other mineral oil treatment combination with a combination of sulfur and phosphorous acid would suppress Microdochium patch and result in acceptable turfgrass quality compared to previous experiments where turfgrass quality was not acceptable when mineral oil was applied every two weeks. Based on preliminary results from the first experiment, notably a loss in green cover percentage from applications of the combination of mineral oil and sulfur, the second experiment began one year later and excluded mineral oil and sulfur combinations. The primary objective of this second experiment was to assess the exclusion of the use of mineral oil in the coldest parts of the winter and to compare application frequencies of every two or three weeks using mineral oil, sulfur, and phosphorous acid regarding Microdochium patch suppression while maintaining acceptable

turfgrass quality. The hypothesis of this experiment was that decreasing mineral oil applications in the winter months would result in greater turfgrass quality compared to more frequent mineral oil applications. The treatment list was set up to explore the number of months necessary to eliminate mineral oil applications that would still suppress Microdochium patch while maintaining turfgrass quality.

Materials and methods

Experimental design

Two separate field experiments were conducted in Corvallis, OR on an annual bluegrass research green at the Lewis-Brown horticulture farm (latitude: 44.550472, longitude: -123.215512). The first experiment focused on comparing two-week rotations in order to assess Microdochium patch suppression, turfgrass quality, and green cover percentage. The second experiment omitted mineral oil and sulfur combinations and focused on testing the exclusion of mineral oil in the coldest parts of the winter as well as compared applications at two- and three-week intervals. Both experiments took place on a putting green that was constructed using 30 cm of USGA recommended particle size sand (USGA, 2018) placed directly onto the native Malabon silty clay loam soil (UC Davis, 2020). Annual bluegrass was established in 2009 using a sand-based sod (Boss Sod, Canada) that was placed onto the constructed sand root zone. Microdochium patch was naturally occurring in both trials and neither experiment was inoculated with *M. nivale*.

The first field experiment took place from 21 September 2015 to 07 April 2016 and was repeated in a different area of the same green from 22 September 2016 to 07 April 2017. The trial consisted of 4 treatments arranged as a randomized complete block design with four

replications with an individual plot size of 1.5 m^2 and a total experiment area of 24 m^2. The four products in the experiment consisted of the mineral oil, Civitas Turf Defense (HollyFrontier Specialty Products, Mississauga, Ontario) that includes a copper (Cu) II pthalocyanine green pigment, applied at 19.94 kg ha^{-1} (27 L ha^{-1}), Sulfur DF (Wilbur-Ellis, San Francisco, CA) applied at 12.2 kg S ha^{-1}, phosphorous acid applied at 3.7 kg H_3PO_3 ha^{-1} as GRIGG P-K Plus (Brandt Consolidated, Springfield, IL) with a fertilizer formulation of 3-5-17, and a non-treated control. The four rotation treatments consisted of: 1) mineral oil applied in combination with phosphorous acid rotated every two weeks with sulfur and phosphorous acid, 2) mineral oil applied in combination with sulfur rotated every two weeks with a sulfur and phosphorous acid combination 3) a combination of sulfur and phosphorous acid applied every two weeks, 4) and a non-treated control. A list of the four treatments can be found in table 2.2.

The second field experiment was conducted from 29 Sep 2016 to 27 Apr 2017 and was repeated in a different area of the same green from 28 Sep 2017 to 27 Apr 2018. The trial consisted of 16 treatments arranged as a randomized complete block design with four replications. The individual plot size was 1.5 m^2 and the total experimental area was 96 m^2. Treatments included seasonal rotations and application intervals of the mineral oil Civitas Turf Defense applied at 19.94 kg ha^{-1} (27 L ha^{-1}), Sulfur DF applied at 12.2 kg S ha^{-1}, phosphorous acid applied at 3.7 kg H_3PO_3 ha^{-1} as Duraphite 12 (J.R. Simplot, Boise, ID) with a fertilizer formulation of 0-0-12, a fungicide rotation control, and a non-treated control. In this experiment, different treatment rotations applied every two or three weeks were compared that excluded mineral oil over different months in the winter. When a combination of mineral oil and phosphorous acid was not applied, a combination of sulfur and phosphorous acid was used. A fungicide rotation control applied every four weeks and a non-treated control were also included.

The list of the sixteen treatments can be found in table 2.4. All treatments were applied using a CO_2 pressurized backpack sprayer equipped with a handheld boom and four XR80015 nozzles (TeeJet, Springfield, IL) delivering 814 L ha^{-1} at 280 kPa at the boom. Displacement speed was calibrated using a metronome.

Turfgrass maintenance

In the first experiment, urea was added to each spray treatment to account for the nitrogen applied with the phosphorous acid product for a total nitrogen input of 4.9 kg N ha^{-1} every two weeks, with the exception in year two when 24.5 kg N ha^{-1} was applied on 07 October 2016 to assist with recuperation from a September hollow-tine aerification. Only the nitrogen in the phosphorous acid product was accounted for in this experiment. In year one of the first experiment, flutolanil [N-[3-(1-methylethoxy)phenyl]-2-(trifluoromethyl)benzamide] was applied on 29 September 2015 because of the presence of cool season brown patch (*Ceratobasidium cereale* D. Murray & L.L. Burpee) and boscalid [3-pyridinecarboxamide, 2-chloro-N-(4´-chloro(1,1´-biphenyl)-2-yl)] was applied to suppress a dollar spot outbreak on 19 October 2015. Research has shown that Microdochium patch is not effectively suppressed by flutolanil (Settle et al., 2011) or boscalid (Popko et al., 2016). In the second experiment, because the phosphorous acid product did not contain any nitrogen, the green received 4.9 kg N ha^{-1} every two weeks in the form of urea sprayed over the entire green, with the exception that in year one 24.5 kg N ha^{-1} was applied on 07 October 2016 and 36.75 kg N ha^{-1} was applied on 05 October 2017 to assist with recuperation from a September hollow-tine aerification. In the first experiment, total nitrogen applied during the trial period was 83 and 78 kg N ha^{-1} in the first and second year respectively. In the second experiment, total nitrogen applied during the trial period

was 88 and 86 kg N ha^{-1} in the first and second year respectively. During the trial period in both experiments, the green was mown at 3.8 mm once a week with the clippings removed. Golfer traffic replication representing 73 golf rounds a day (average winter play at a local municipal course in Corvallis, OR) occurred on both trials by walking over the plots 5 days a week with golf shoes following the protocol outlined by Hathaway and Nikolai (2005). In addition, sethoxydim [2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one] was applied in the summer to suppress creeping bentgrass.

Response variables

Because of turfgrass thinning, digital image analysis was made complicated to accurately assess Microdochium patch percentage. Microdochium patch percentage was, therefore, quantified using stratified sampling (Laycock and Canaway, 1980; Richarson et al., 2001) with a 100-point grid that was overlaid onto digital images that were collected using a Sony DSC-H9 camera that was mounted onto an enclosed light box equipped with four battery-powered 40-W spring lamps. Digital grid overlays were added using Sigma Scan Pro version 5.0 (Systat Software, Inc. San Jose, CA). The disease percentage data were then used to construct area under disease progress curves (AUDPC) using the trapezoidal method (Shaner and Finney, 1977) from the beginning of the trial up to the peak of disease. In the first experiment, in order to quantify discoloration of the experimental units caused by the treatments, green cover percentage was assessed on the images corresponding to the three treatments (the non-treated control was excluded because of the presence of excessive Microdochium patch damage). The Turf Analyzer program (Karcher and Richardson, 2013; Turf Analyzer, 2020) with the following settings: hue 75 to 360, saturation 45 to 100, and brightness 0 to 75 was used for the digital image analysis.

These setting were chosen to capture green pixels and exclude pixels exhibiting disease symptoms and discoloration caused by the treatments (Figure 2.1). Attempts to use digital image analysis for the second experiment were not possible because of some residual damage from the summer disease anthracnose (*Colletotrichum cereale* Manns). For this reason, annual bluegrass abiotic damage percentage was estimated visually. Turfgrass quality ratings were assigned using the National Turfgrass Evaluation Program (1 to 9 scale where 9 is the highest quality and a rating of 6 is considered minimally acceptable).

Statistical analyses

Statistical analysis of disease percentage at the peak of disease, AUDPC, and turfgrass quality were carried out using R 3.5.2 (R Core Team, 2018). Because of the lack of disease for multiple AUDPC and disease percentage data points, the assumption of homogeneous variance could not be met, even after attempts of data transformation. For these reasons, a permutation test was performed using the disease percentage and AUDPC data by running one million simulations to test the probability of observing an F-statistic as extreme or more extreme than the F-statistic observed (P-value) (Ramsey and Shafer, 2002). A permutation test provides the probability of the results observed being caused by random chance. Turfgrass quality data, being ordinal in nature (Karcher, 2000), was analyzed using a Kruskal-wallis one-way analysis of variance and a Dunn's test (Dunn, 1964) was used to separate individual means. Green cover percentage data were analyzed using repeated measures analysis of variance, standard t-tests were used to make multiple comparisons, and family-wise errors were corrected using the Holm's method (Holm, 1979) using Proc Mixed in SAS 9.4 (SAS Institute Inc., Cary, N.C).

Results

Experiment one: two-week rotations

Disease percentage and AUDPC

In each year of the first experiment, all three treatments resulted in less than 1% average Microdochium patch compared to an average Microdochium patch of 46% in 2016 and 55% in 2017 in the non-treated control (Figures 2.2 and 2.3). The results of the permutation test revealed a $P < 0.001$ for the peak of disease in both years and a $P < 0.001$ for the AUDPC in both years. These permutation tests provide strong evidence that the Microdochium patch suppression observed by the three treatments was not because of random chance (i.e. the suppression of Microdochium patch was a result of the experimental treatments).

Effects on green cover percentage

The repeated measures analysis indicated a significant interaction between treatment and month ($P < 0.001$) in both years. The mineral oil and phosphorous acid combination rotated with the sulfur and phosphorous acid combination was always in the group with the highest green cover percentage in every month in both years of the experiment. The sulfur and phosphorous acid combination applied every two weeks had the lowest green cover percentage on 09 December 2015 and in all months analyzed from November 2016 to February 2017. On the 18 January 2016, there was a reduction in green cover percentage caused by abiotic damage for the combination of mineral oil and sulfur rotated with sulfur and phosphorous acid (Figure 2.1 and Table 2.1). The same treatment caused a similar, although not as pronounced, reduction in green cover percentage in January 2017.

Turfgrass quality

The mineral oil and phosphorous acid combination applied in rotation with the combination of sulfur and phosphorous acid was the only treatment that was significantly different from the non-treated control throughout the trial (Tables 2.2 and 2.3). However, even though the average turfgrass quality was ≥ 6.0 throughout the trial on plots receiving the aforementioned rotation, on some dates, the turfgrass quality rating on individual plots received an unacceptable turfgrass rating (< 6.0) because of a minor, although unacceptable, loss in acceptable turfgrass color because of abiotic damage from the treatments.

Experiment two: two- and three-week rotation experiment

Disease percentage and AUDPC

At the peak of disease in both years, Microdochium patch was absent from all plots that received treatments every two weeks with the exception of the combination of sulfur and phosphorous acid applied every two weeks. The average Microdochium patch percentage at the peak of disease was less than 4% for the remaining treatments compared to 49.6% and 64.6% in the non-treated control in both years respectively. The AUDPC data followed the same trends as the disease percentage data, except some treatments applied every two weeks had an AUDPC greater than zero when at the peak of disease there was no disease present. This is explained because there was a small amount of disease (less than 1% on average) that was present in November 2017, but the damaged areas had recuperated by the peak of disease (Table 2.4).

The permutation test revealed a $P < 0.001$ for the peak of disease and AUDPC in both years. These permutation tests provide strong evidence that the Microdochium patch suppression observed by the treatments was not because of random chance (i.e. the suppression of

Microdochium patch was a result of the experimental treatments). The permutation test provides a high confidence ($P < 0.001$) that all treatments tested suppressed Microdochium patch compared to the non-treated control although these analyses do not provide the means of inferring differences among the different rotation treatments or the fungicide control.

Annual bluegrass thinning

The area affected by annual bluegrass thinning was most expressed in the month of January in both years and never exceeded an average of 2% for the treatments. No significant differences were found among the treatments, although of note was the observation that only rotations that included mineral oil expressed annual bluegrass thinning. Of those treatments, rotations every three weeks that did not include mineral oil in December, did not exhibit annual bluegrass thinning on the January rating date for either year (Table 2.5).

Turfgrass quality

Only two treatments received acceptable turfgrass quality ratings throughout both years of the study. One of those treatment rotations was on plots receiving a combination of mineral oil and phosphorous acid in October, November, March, and April with a combination of sulfur and phosphorous acid applied from December through February every two weeks. The other treatment receiving acceptable turfgrass quality ratings was identical to the precedent treatment with the exception that in December, the mineral oil and phosphorous acid combination was applied instead of the sulfur and phosphorous acid combination (Tables 2.6 and 2.7).

Discussion

This research supports the previous findings that sulfur and phosphorous acid combinations suppress Microdochium patch when applied every two weeks in Western Oregon (Mattox et al., 2020) and also demonstrate that a three-week application interval suppresses Microdochium patch throughout the fall and winter. These findings are similar to results observed in Scandinavia where a three-week application interval from August through November of mineral oil and phosphorous acid suppressed Microdochium patch (Aamlid et al., 2018). Current fungicide application intervals recommended for turfgrass management vary, with typical fungicide labels listing an interval between 7 and 28 days (Clarke et al., 2019). Potential advantages of increasing the time between applications of the same rate would be to; decrease total cost of product and labor application costs, decrease the environmental impact compared to more frequent applications (Kovach et al., 1992), reduce exposure to pesticides by pesticide handlers (Environmental Protection Agency, 2020), and to decrease the total sulfur applications in order to decrease the risk of anthracnose on annual bluegrass putting greens (McDonald et al., 2018) and the development of black layer (Berndt and Vargas, 2008). Potential disadvantages would be the lack of complete disease suppression by three-week applications that may not meet golfer expectations (Walsh, 2005).

It is unknown why some of the rotations applied on a two-week application frequency in this study completely suppressed Microdochium patch while the same rotations on a three-week application frequency failed to completely suppress disease. Applications of the mineral oil used in this experiment are reported to cause an induced systemic response in turfgrass (Cortes-Barco et al., 2010a and 2010b). In a greenhouse study where creeping bentgrass (*Agrostis stoloniferous*) received a mineral oil injection into the sand substrate and was inoculated 7 days

after treatment with *M. nivale* grown on wheat bran, Microdochium patch was suppressed compared to the non-treated control up to 10 days after inoculation, however disease percentage did continuously increase at each rating interval on the mineral oil treated plants (Cortes-Barco et al., 2010a), suggesting that more frequent applications may be necessary to suppress Microdochium patch to acceptable levels. In the same study, growth media amended with the mineral oil reduced the growth rate of *M. nivale in vitro* up to seven days, however the effects were diminished after 10 days suggesting that the mechanism behind the inhibition was reduced (Cortes-Barco et al., 2010a). These findings suggest that the mode of action of the mineral oil on disease suppression may lose its effect over time. It is unclear if rates greater than 19.9 kg mineral oil ha^{-1} would suppress Microdochium patch for longer periods. A study that included 10.0 and 19.9 kg mineral oil ha^{-1} applied in combination with different rates of sulfur or phosphorous acid every two weeks demonstrated that both rates suppressed Microdochium patch (Chapter 1 of this dissertation). In that experiment, applications that included 19.9 kg mineral oil in combination with phosphorous acid were free of Microdochium patch compared to the 10.0 kg mineral oil ha^{-1} rate with some Microdochium patch present (less than 2%) (Chapter 1 of this dissertation). A similar observation occurred in Scandinavia comparing 19.9 kg (27 L ha^{-1}) and 39.8 kg (54 L ha^{-1}) of mineral oil ha^{-1} applied every three weeks, where there was no statistical difference in Microdochium patch suppression between the treatments, although there was a trend for less overall disease observed where the higher rate was applied (Aamlid et al., 2018).

Research suggests that the mode of action of phosphorous acid on the suppression of Microdochium patch is multi-faceted. Phosphorous acid has been shown to have a fungistatic effect on *M. nivale in vitro*, to have led to a decrease in conidial germination, as well as to have been shown to increase phytoalexin production in annual bluegrass (Dempsey, 2016).

Phosphorous acid concentration in annual bluegrass leaves has been shown to reduce over time with a maximum leaf concentration reported to be 48 hours after application when recorded every 6 hours and after one week when recorded on a weekly basis in both February and July. Specifically, the phosphorous acid leaf concentration declined steadily after one week, with a 73% reduction after four weeks in February and more than 50% reduction after three weeks in July (Dempsey, 2016). The findings suggest that, like mineral oil, phosphorous acid will likely need to be applied frequently to suppress Microdochium patch.

Additionally, application intervals for traditional turfgrass fungicides usually range from 7 to 28 days and multiple avenues of why there is a decrease in fungicide residues is suspected (Latin, 2011). Rainfall (Koch et al., 2015), microbial degradation (Magri and Haith, 2009), removal of leaf tissue (and with it the product), sorption of the products to the leaves and thatch (Sigler et al., 2003), and turfgrass metabolism of the fungicides are all possible avenues. These reasons may provide an explanation as to why there was a difference in Microdochium patch suppression between two- and three-week application frequencies in this current trial.

The loss of green cover percentage observed in the first experiment on plots receiving a combination of mineral oil and sulfur is similar to another study in Western Oregon focusing on the use of different rates of mineral oil, sulfur, and phosphorous acid, where mineral oil and sulfur combinations applied every two weeks resulted in a consistent loss of green cover and resulted in turfgrass quality considered unacceptable for golf course putting greens (Chapter 1 of this dissertation). This current experiment was subjected to abiotic stress in the form of replicated golfer traffic and similar to findings in a previous study that included mineral oil applications exposed to abiotic stress in the form of rolling five days a week (Mattox et al.,

2020), the plots receiving mineral oil applications experienced the most abiotic damage, notably expressed as a decrease in green cover percentage.

It is unclear why mineral oil and sulfur combinations result in a decrease in green cover percentage, although mineral oils have been shown to lead to stomatal occlusion in turfgrass (Kreuser and Rossi, 2014). It is also suspected that a decrease in gas exchange by mineral oil applications resulted in a loss of photosynthesis in grape leaves (Finger et al., 2002). Specific to combining mineral oil and sulfur, a decrease in photosynthesis in apple leaves following an application of mineral oil combined with sulfur has also been reported (Ferree et al., 1999). It is unclear why the combination of mineral oil and sulfur leads to a greater loss in green cover percentage compared to mineral oil and phosphorous acid, although it was speculated that the light intensity striking apple leaves was affected by sulfur applications (Hyre, 1939), whereas this may not be the case with phosphorous acid applications. If there is a loss of photosynthetic activity resulting from sulfur applications, this may explain to some extent the loss of green cover percentage occurring only transiently during the winter months when daylengths are shorter and temperatures are reduced. Photosynthesis may also play an important role in providing energy to plants that are undergoing a priming of plant defenses where carbon allocation may be going to induced resistance costs leaving a deficit for growth (Heil and Baldwin, 2002; Vos et al., 2013). These negative effects may increase in the colder months of the winter when daylengths are shorter and temperatures are lower than more favorable growth conditions for annual bluegrass (Vargas and Turgeon, 2004).

It was noted that the loss in green cover percentage was greater in the first year of the experiment (2015-2016) compared to the second year (2016-2017) on treatments that included the mineral oil and sulfur combinations even though the mean temperatures overall were warmer

in the first year compared the second year. One observation that may have played a role is that the minimum temperature decreased below freezing earlier in the first year compared to the second year (Table 2.8). In November 2015, there were seven days when the minimum temperature dipped below freezing starting on 22 November 2015 with the minimum temperature of – 6.6°C recorded on 30 November 2015. In contrast, the first date when temperatures dipped below freezing in the second year was on 07 December 2016. Photosynthetic activity has been reported as low as - 4°C in grasses (Skinner, 2007; Höglind et al., 2011), and the minimum temperature required for photosynthesis is reported to be when the leaf tissue water is not frozen (Pisek, 1973). It is possible that this earlier freezing event caused a reduction in photosynthetic activity and this may have played a role in the photosynthetic activity of the annual bluegrass. Because sulfur and mineral oil combinations were shown to consistently reduce turfgrass quality and because sulfur applications have been tied to an increase in the risk of anthracnose (McDonald et al., 2018), these data would suggest that mineral oil applied in combination with sulfur is not an effective means of suppressing Microdochium patch while maintaining acceptable turfgrass quality in Western Oregon.

Turfgrass quality ratings in the second experiment were affected by Microdochium patch suppression, turfgrass thinning, and turfgrass color and only two treatments resulted in an average turfgrass quality rating considered acceptable in both years of the experiment. In areas where fungicide restrictions occur, turfgrass managers may accept more damage from disease or abiotic damage than where fungicides are permitted, however it is likely that some turfgrass managers will continue to require disease free turfgrass surfaces to meet golfer expectations on certain golf courses (Walsh, 2005).

Conclusion

These field experiments demonstrate that mineral oil, sulfur, and phosphorous acid suppress Microdochium patch compared to a non-treated control and that reducing the frequency of mineral oil in the winter months shows promise for maintaining acceptable turfgrass quality. Of concern is the continual incidence of abiotic damage expressed as a loss of green cover percentage. In the future, suppression of Microdochium patch accompanied with a minimal loss of acceptable winter turfgrass quality may be a welcome option for turfgrass managers with limited pesticide options. Future research is warranted to determine any long-term negative impacts of winter mineral oil, sulfur, and phosphorous acid applications, specifically regarding impacts on summer pathogens such as anthracnose, a change in soil pH, or nutrient concerns such as phosphorous availability or copper additions.

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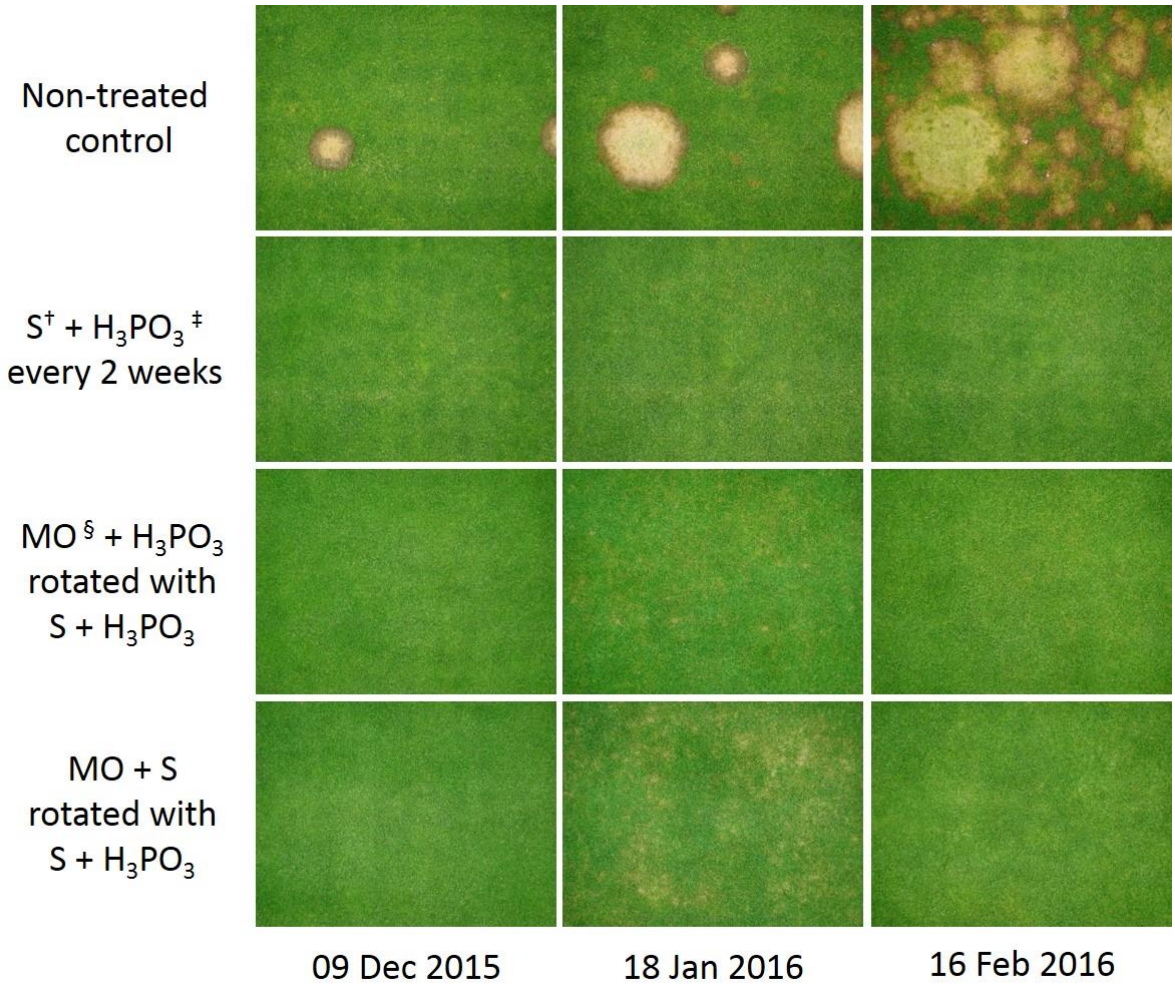

Figure 2.1. Light box photo images of plots from 09 Dec 2015 to 16 Feb 2016. † S = Sulfur treatments applied at 12.2 kg ha^{-1} ‡ H_3PO_3 = phosphorous acid applied at 3.7 kg H_3PO_3 ha^{-1} § MO = Mineral oil treatments of Civitas Turf Defense applied at 19.94 kg ha^{-1}

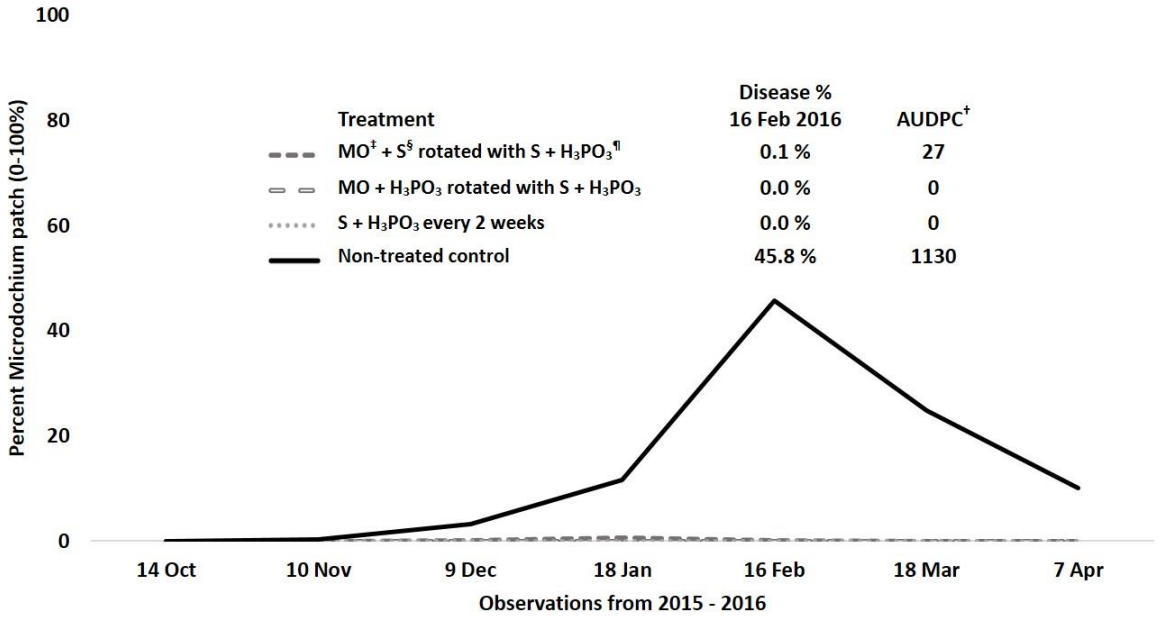

Figure 2.2. Effects of treatments on area under disease progress curve and disease percentage on an annual bluegrass putting green in Corvallis, OR. † Calculated from the beginning of the trial in year one (21 Sep. 2015) until the peak of disease (16 Feb 2016). ‡ MO = Mineral oil treatments of Civitas Turf Defense applied at 19.94 kg ha^{-1} § S = Sulfur treatments applied at 12.2 kg ha^{-1} ¶ H_3PO_3 = phosphorous acid applied at 3.7 kg H_3PO_3 ha^{-1} # Means in the same column followed by the same letter are not statically significant according to Dunn's all-pairwise comparison test at $P \leq 0.05$.

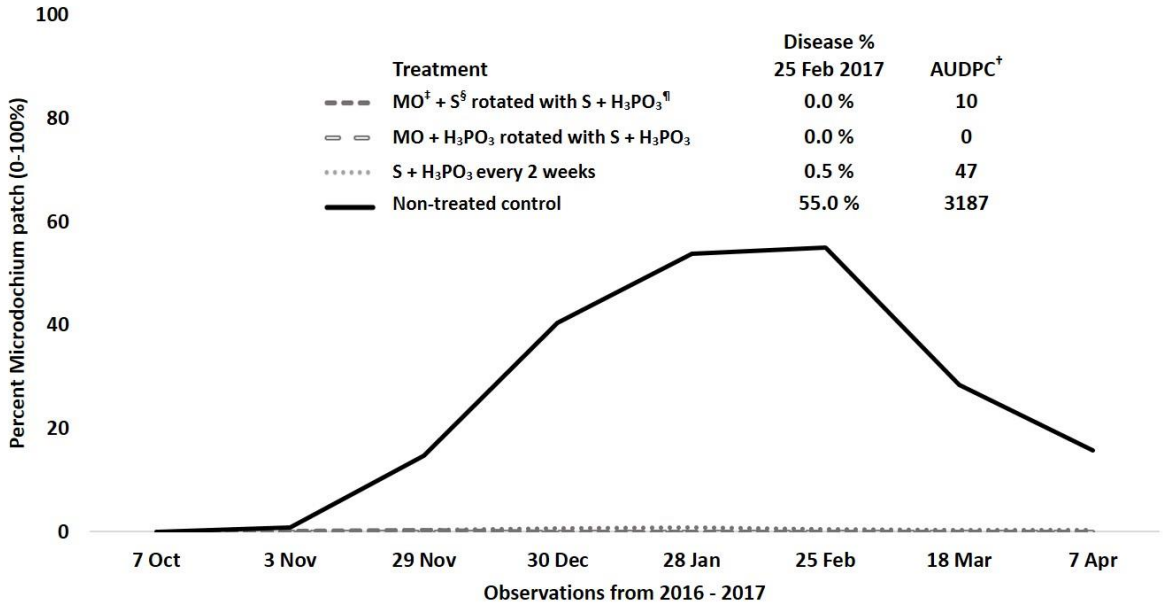

Figure 2.3. Effects of treatments on area under disease progress curve and disease percentage on an annual bluegrass putting green in Corvallis, OR. † Calculated from the beginning of the trial in year two (22 Sep. 2016) until the peak of disease (25 Feb 2017). ‡ MO = Mineral oil treatments of Civitas Turf Defense applied at 19.94 kg ha^{-1} § S = Sulfur treatments applied at 12.2 kg ha^{-1} ¶ H_3PO_3 = phosphorous acid applied at 3.7 kg H_3PO_3 ha^{-1} # Means in the same column followed by the same letter are not statically significant according to Dunn's all-pairwise comparison test at $P \leq 0.05$.

	< - - - - Green Cover Percentage (2015 to 2016) - - - - >							
Treatment	10 Nov		09 Dec		18 Jan		19 Feb	
MO† + S‡ rotated with S + H_3PO_3§	97.3%	a¶	98.5%	ab	92.1%	c	98.2%	a
MO + H_3PO_3 rotated with S + H_3PO_3	97.4%	a	98.8%	a	98.2%	a	98.4%	a
S + H_3PO_3 every 2 weeks	96.1%	a	98.3%	b	96.2%	b	97.1%	a

	< - - - - Green Cover Percentage (2016 to 2017) - - - - >							
Treatment	29 Nov		30 Dec		28 Jan		25 Feb	
MO + S rotated with S + H_3PO_3	98.6%	a	98.0%	b	96.4%	b	99.1%	b
MO + H_3PO_3 rotated with S + H_3PO_3	98.9%	a	99.1%	a	98.5%	a	99.4%	a
S + H_3PO_3 every 2 weeks	97.1%	b	87.6%	c	92.4%	c	97.4%	c

Table 2.1. Effects of treatments on green cover percentage on an annual bluegrass putting green in Corvallis, OR. † MO = Mineral oil treatments of Civitas Turf Defense applied at 19.94 kg ha^{-1} ‡ S = Sulfur treatments applied at 12.2 kg ha^{-1} § H_3PO_3 = phosphorous acid applied at 3.7 kg H_3PO_3 ha^{-1} ¶ Means in the same column followed by the same letter are not statically different according to standard t-tests at $P \leq 0.05$.

		Kruskal-Wallis ANOVA Year One 2015-2016									
Source of variation	DF	Nov 20		Dec 23		Jan 18		Feb 24		Mar 25	
Treatment	3	P < 0.001		P < 0.001		P < 0.001		P < 0.001		P < 0.001	
MO† + S‡ rotated with S + H_3PO_3§		6.6	ab¶	7.0	a	5.0	ab	6.4	ab	6.0	ab
MO + H_3PO_3 rotated with S + H_3PO_3		7.0	a	7.5	a	6.4	a	7.0	a	7.0	a
S + H_3PO_3 every 2 weeks		7.0	a	6.6	ab	6.3	ab	6.5	ab	5.8	ab
Non-treated control		5.4	b	4.5	b	4.8	b	3.3	b	3.0	b

Table 2.2. Effects of treatments on turfgrass quality ratings on an annual bluegrass putting green in Corvallis, OR. † MO = Mineral oil treatments of Civitas Turf Defense applied at 19.94 kg ha^{-1} ‡ S = Sulfur treatments applied at 12.2 kg ha^{-1} § H_3PO_3 = phosphorous acid applied at 3.7 kg H_3PO_3 ha^{-1} ¶ Means in the same column followed by the same letter are not statically significant according to Dunn's all-pairwise comparison test at $P \leq 0.05$.

		Kruskal-Wallis ANOVA Year Two 2016-2017									
Source of variation	DF	Nov 28		Dec 23		Jan 29		Feb 25		Mar 31	
Treatment	3	P = 0.006		P = 0.004		P < 0.001		P < 0.001		P < 0.001	
MO† + S‡ rotated with S + H_3PO_3§		6.8	a¶	6.8	a	5.0	ab	7.0	a	6.8	ab
MO + H_3PO_3 rotated with S + H_3PO_3		6.6	ab	6.8	a	6.0	a	7.0	a	8.0	a
S + H_3PO_3 every 2 weeks		6.5	ab	6.0	ab	5.6	ab	5.6	ab	5.8	ab
Non-treated control		5.0	b	3.8	b	2.8	b	3.0	b	3.0	b

Table 2.3. Effects of treatments on turfgrass quality ratings on an annual bluegrass putting green in Corvallis, OR. † MO = Mineral oil treatments of Civitas Turf Defense applied at 19.94 kg ha^{-1} ‡ S = Sulfur treatments applied at 12.2 kg ha^{-1} § H_3PO_3 = phosphorous acid applied at 3.7 kg H_3PO_3 ha^{-1} ¶ Means in the same column followed by the same letter are not statically significant according to Dunn's all-pairwise comparison test at $P \leq 0.05$.

	Treatment							Year One: 2016 - 2017		Year Two: 2017 - 2018	
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	28 Jan 17	AUDPC	14 Feb 18	AUDPC
- - Every 2 weeks - -	MO† + PA‡		S§ + PA			MO + PA		0.0 %	0	0.0 %	32
	MO + PA			S + PA		MO + PA		0.0 %	0	0.0 %	10
	MO + PA			S + PA			MO + PA	0.0 %	0	0.0 %	0
	MO + PA		S + PA				MO + PA	0.0 %	0	0.0 %	0
	MO + PA rotated with S + PA							0.0 %	0	0.0 %	53
	MO + PA							0.0 %	0	0.0 %	0
	S + PA							1.8 %	96	1.0 %	43
- - Every 3 weeks - -	MO + PA		S + PA			MO + PA		0.9 %	64	2.5 %	270
	MO + PA			S + PA		MO + PA		0.6 %	17	2.3 %	194
	MO + PA			S + PA			MO + PA	1.1 %	50	0.5 %	64
	MO + PA		S + PA				MO + PA	0.3 %	15	3.6 %	211
	MO + PA rotated with S + PA							3.8 %	205	0.1 %	5
	MO + PA							2.1 %	134	0.0 %	13
	S + PA							7.0 %	290	6.3 %	393
Fungicide control (every 4 weeks)								0.0 %	0	0.1 %	14
Non-treated control								49.6 %	2273	64.6 %	2185

Table 2.4. Effects of treatments on disease percentage at peak of disease and on area under diseases progress curve (AUDPC) on an annual bluegrass putting green in Corvallis, OR. † MO = Mineral oil treatments of Civitas Turf Defense applied at 19.94 kg ha^{-1} ‡ PA = phosphorous acid applied at 3.7 kg H_3PO_3 ha^{-1} § S = Sulfur treatments applied at 12.2 kg ha^{-1}

	Source of variation							DF	Annual Bluegrass Thinning			
	Treatment							15	P=0.082		P=0.251	
	Oct	Nov	Dec	Jan	Feb	Mar	Apr		28 Jan 2017		20 Jan 2018	
- - Every 2 weeks - -	MO† + PA‡		S§ + PA			MO + PA			1.0%	a¶	0.1%	a
	MO + PA			S + PA		MO + PA			0.6%	a	1.3%	a
	MO + PA			S + PA			MO + PA		1.1%	a	1.2%	a
	MO + PA		S + PA				MO + PA		1.3%	a	0.0%	a
	MO + PA rotated with S + PA								0.4%	a	0.2%	a
	MO + PA								0.7%	a	1.8%	a
	S + PA								0.0%	a	0.0%	a
- - Every 3 weeks - -	MO + PA		S + PA			MO + PA			0.0%	a	0.0%	a
	MO + PA			S + PA		MO + PA			0.8%	a	0.8%	a
	MO + PA			S + PA			MO + PA		0.5%	a	1.2%	a
	MO + PA		S + PA				MO + PA		0.0%	a	0.0%	a
	MO + PA rotated with S + PA								0.6%	a	0.0%	a
	MO + PA								0.0%	a	0.9%	a
	S + PA								0.0%	a	0.0%	a
Fungicide control (every 4 wks)									0.0%	a	0.0%	a
Non-treated control									0.0%	a	0.0%	a

Table 2.5. Effects of treatments on visual estimates of annual bluegrass thinning on an annual bluegrass putting green in Corvallis, OR. † MO = Mineral oil treatments of Civitas Turf Defense applied at 19.94 kg ha^{-1} ‡ PA = phosphorous acid applied at 3.7 kg H_3PO_3 ha^{-1} § S = Sulfur treatments applied at 12.2 kg ha^{-1} ¶ Means in the same column followed by the same letter are not statically significant according to Dunn's all-pairwise comparison test at $P \leq 0.05$.

	Source of variation							DF	Turfgrass Quality 2016 - 2017													
	Treatment							15	P=.004†		P<.001		P=.002		P=.012		P<.001		P<.001	
	Oct	Nov	Dec	Jan	Feb	Mar	Apr		29 Nov		30 Dec		28 Jan		25 Feb		31 Mar		27 Apr	
- - - - Every 2 weeks - - - -	MO‡ + PA§		S¶ + PA			MO + PA			7.8	a#	7.3	a	6.0	a	7.0	a	7.5	abc	7.0	abc
	MO + PA			S + PA		MO + PA			7.5	ab	7.0	a	6.1	a	6.5	ab	8.0	a	7.3	abc
	MO + PA			S + PA			MO + PA		7.8	a	7.0	a	5.9	ab	6.4	ab	6.8	abc	6.5	abc
	MO + PA		S + PA				MO + PA		7.3	ab	7.0	a	5.8	ab	6.1	ab	6.1	abc	7.0	abc
	MO + PA rotated with S + PA								7.5	ab	7.0	a	6.3	a	7.0	a	8.0	a	7.0	abc
	MO + PA								7.0	ab	6.4	ab	5.8	ab	6.5	ab	7.8	ab	7.8	a
	S + PA								5.9	ab	5.8	ab	5.4	ab	5.6	ab	5.5	abc	5.4	bc
- - - - Every 3 weeks - - - -	MO + PA		S + PA			MO + PA			5.4	ab	5.3	ab	5.3	ab	6.1	ab	7.0	abc	7.0	abc
	MO + PA			S + PA		MO + PA			6.3	ab	6.0	ab	5.6	ab	6.1	ab	7.5	abc	7.5	ab
	MO + PA			S + PA			MO + PA		7.1	ab	6.0	ab	5.4	ab	5.9	ab	6.6	abc	7.0	abc
	MO + PA		S + PA				MO + PA		6.9	ab	6.0	ab	5.5	ab	5.9	ab	6.0	abc	7.0	abc
	MO + PA rotated with S + PA								5.9	ab	5.4	ab	5.1	ab	5.5	ab	5.9	abc	6.6	abc
	MO + PA								6.0	ab	5.6	ab	5.1	ab	5.5	ab	6.8	abc	7.3	abc
	S + PA								5.6	ab	5.3	ab	5.1	ab	4.9	ab	5.1	bc	5.5	bc
	Fungicide control (every 4 weeks)								6.4	ab	6.3	ab	5.9	ab	6.6	ab	6.5	abc	6.5	abc
	Non-treated control								4.5	b	3.9	b	3.5	b	2.5	b	2.8	c	3.0	c

Table 2.6. Effects of treatments on turfgrass quality on an annual bluegrass putting green in Corvallis, OR. † P-values are Bonferroni adjusted across dates ‡ MO = Mineral oil treatments of Civitas Turf Defense applied at 19.94 kg ha^{-1} § PA = phosphorous acid applied at 3.7 kg H_3PO_3 ha^{-1} ¶ S = Sulfur treatments applied at 12.2 kg ha^{-1} # Means in the same column followed by the same letter are not statically significant according to Dunn's all-pairwise comparison test at $P \leq 0.05$.

	Source of variation							DF	Turfgrass Quality 2017 - 2018													
	Treatment							15	p=ns†		p=.005		p=.018		p<.001		p<.001		p<.001	
	Oct	Nov	Dec	Jan	Feb	Mar	Apr		16 Nov		16 Dec		14 Jan		14 Feb		15 Mar		12 Apr	
- - - - Every 2 weeks - - - -	MO‡ + PA§		S¶ + PA			MO + PA			6.8	a#	6.8	ab	6.5	ab	6.8	ab	7.3	ab	7.8	a
	MO + PA			S + PA		MO + PA			6.9	a	6.9	ab	6.8	ab	8.0	a	8.0	a	8.0	a
	MO + PA			S + PA			MO + PA		8.0	a	8.0	a	6.8	ab	7.8	a	8.0	a	6.8	ab
	MO + PA		S + PA				MO + PA		7.1	a	7.4	ab	6.5	ab	7.8	a	7.3	ab	6.8	ab
	MO + PA rotated with S + PA								6.1	a	6.1	ab	5.4	ab	6.3	ab	6.9	ab	7.5	ab
	MO + PA								8.0	a	7.8	a	7.0	a	8.0	a	8.0	a	8.0	a
	S + PA								6.4	a	5.9	ab	5.9	ab	5.6	ab	5.9	ab	5.6	ab
- - - - Every 3 weeks - - - -	MO + PA		S + PA			MO + PA			6.1	a	6.1	ab	5.8	ab	5.3	ab	5.3	ab	5.5	ab
	MO + PA			S + PA		MO + PA			5.6	a	5.3	ab	5.5	ab	5.4	ab	5.9	ab	6.5	ab
	MO + PA			S + PA			MO + PA		6.8	a	6.3	ab	5.9	ab	6.1	ab	6.8	ab	6.6	ab
	MO + PA		S + PA				MO + PA		6.1	a	5.8	ab	6.4	ab	6.4	ab	6.1	ab	5.9	ab
	MO + PA rotated with S + PA								7.5	a	6.8	ab	7.3	a	7.8	a	7.5	ab	7.8	a
	MO + PA								7.4	a	6.9	ab	6.6	ab	7.8	a	8.0	a	8.0	a
	S + PA								5.4	a	5.4	ab	5.1	ab	5.0	ab	5.0	ab	5.1	ab
Fungicide control (every 4 weeks)									6.8	a	6.1	ab	6.1	ab	6.9	ab	7.5	ab	7.5	ab
Non-treated control									5.4	a	4.6	b	3.8	b	2.8	b	3.0	b	3.0	b

Table 2.7. Effects of treatments on turfgrass quality on an annual bluegrass putting green in Corvallis, OR. † P-values are Bonferroni adjusted across dates ‡ MO = Mineral oil treatments of Civitas Turf Defense applied at 19.94 kg ha^{-1} § PA = phosphorous acid applied at 3.7 kg H_3PO_3 ha^{-1} ¶ S = Sulfur treatments applied at 12.2 kg ha^{-1} # Means in the same column followed by the same letter are not statically significant according to Dunn's all-pairwise comparison test at $P \leq 0.05$.

	2015-2016 Lewis-Brown Farm				2016-2017 Lewis-Brown Farm				2017-2018 Lewis-Brown Farm			
	Mean temp	+/- 30 yr mean†	Min temp	Max temp	Mean temp	+/- 30 yr mean†	Min temp	Max temp	Mean temp	+/- 30 yr mean†	Min temp	Max temp
September	15.8	-1.0	3.6	32.6	15.9	-1.0	3.6	31.1	17.5	0.6	5.2	35.8
October	14.0	2.3	2.9	27.2	12.2	0.5	2.3	22.5	11.3	-0.5	0.1	23.1
November	6.5	-0.9	-6.6	17.3	9.9	2.5	2.5	20.3	7.9	0.6	-0.4	17.2
December	6.3	2.1	-3.1	16.1	2.6	-1.6	-6.8	11.9	3.0	-1.2	-5.7	13.5
January	5.4	0.6	-5.7	16.7	1.8	-3.0	-9.6	12.0	6.5	1.7	-2.2	17.0
February	8.1	2.1	-0.5	16.7	5.5	-0.5	-1.5	15.6	5.4	-0.6	-5.0	17.3
March	9.0	0.8	0.6	21.5	8.4	0.3	0.2	18.2	6.8	-1.4	-1.5	20.3
April	12.3	2.2	0.9	29.8	9.5	-0.6	0.1	19.5	10.4	0.3	-0.5	27.1

Table 2.8.Weather data summary for the Lewis-Brown Turfgrass Research Farm in Corvallis, OR from September 2015 to April 2016, September 2016 to April 2017, and September 2017 to April 2018. † Thirty-year mean monthly data collected on 04 February 2020 from the National Oceanic and Atmospheric Administration (https://www.ncdc.noaa.gov/cdo-web/datatools/normals).

Chapter 3. The influence of iron sulfate application interval on the suppression of Microdochium patch on an annual bluegrass research green in Western Oregon

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Microdochium patch occurs in cool and humid environments (Dwyer, 2017) in the presence of the fungal pathogen *Microdochium nivale* (Fries) Samuels & I.C. Hallett on a susceptible host such as annual bluegrass (*Poa annua* L. f. reptans (Hauskins) T. Koyama). In areas meeting these conditions, like in the Pacific Northwest, Microdochium patch is considered the most important turfgrass disease (Vargas, 2005). The majority of golf courses in these areas apply fungicides to mitigate the damage caused by Microdochium patch, although legislation makes it a challenge for some turfgrass managers to use fungicides (Christie, 2010).

Recent research has demonstrated that iron sulfate heptahydrate fertilizer can suppress Microdochium patch on annual bluegrass putting greens although the most effective rate (2 lbs. $FeSO_4 \cdot 7H_2O$ per 1,000 sq. ft. applied in a carrier volume of 2 gal per 1,000 sq. ft. every 2 weeks) resulted in unacceptable turfgrass quality because of turfgrass thinning (Mattox et al., 2016). It is unknown if application intervals longer than every 2 weeks would be effective to suppress Microdochium patch or allay any negative effects of turfgrass thinning previously observed in other studies. The objective of this study was to quantify the effects of application intervals of $FeSO_4 \cdot 7H_2O$ applied at 2 lbs. per 1,000 sq. ft. on the suppression of Microdochium patch and turfgrass quality on an annual bluegrass research green.

A field trial was conducted on an annual bluegrass research green at the Lewis-Brown Horticulture Farm in Corvallis, OR. The trial took place from 22 October 2015 to 28 April 2016 and was repeated on a different section of the same research green from 21 October 2016 to 22 April 2017. The trial was arranged as a randomized complete block design with four replications and the plot size was 16 sq. ft. There were 5 treatments: a not treated control and iron sulfate heptahydrate treatments applied at 2 lbs. per 1,000 sq. ft. at four intervals: 2, 4, 6, and 8 weeks. A CO_2 pressurized backpack sprayer equipped with a handheld boom and four XR80015 nozzles

(TeeJet, Springfield, IL) delivered 2.5 gal per 1,000 sq. ft. at 40 lb/sq inch. The iron sulfate heptahydrate was applied using 67.6 ounce bottles that were shaken vigorously prior to application and between applications to ensure that the iron sulfate heptahydrate was in suspension. An effective water carrier volume of 5.0 gal per 1,000 sq. ft. was achieved by making two passes over the plot with the sprayer and using a metronome to ensure a consistent application. During the trial, the research green received 0.1 lbs. N per 1,000 sq. ft. of urea (46-0-0) every two weeks and was mown at 0.15 inches with clippings removed. During the summer of 2016, irrigation was applied and anthracnose (*Colletotrichum cereale*) was controlled prophylactically with fungicides.

Monthly disease percentages were quantified using a 100-point grid that was overlaid using Sigma Scan Pro version 5.0 onto digital images that were collected using a Sony DSC-H9 camera mounted onto a light box (Laycock and Canaway, 1980). These monthly disease percentages were used to calculate area under disease progress curves (AUDPC). Turfgrass quality ratings were assigned following the National Turfgrass Evaluation Program (NTEP, 1 to 9 scale where 9 is the highest quality and a rating of 6 is considered minimally acceptable).

Statistical analyses were performed using R 3.5.2 (R Core Team, 2018) and log transformations were used when the data were not homoscedastic. Log transformed data were back-transformed for the construction of figures and tables. Peak of disease and AUDPC data were subjected to analysis of variance and Fisher's Protected Least Significant Difference (LSD) was used at a probability level of 0.05 for a comparison between the means. Data were analyzed separately by year because a year effect ($P < 0.001$) and a year by treatment effect were observed ($P < 0.001$) for the data at the peak of disease and a year effect ($P = 0.032$) was observed for the

AUDPC data. Kruskal-wallis one-way analysis of variance on ranks was used to analyze the ordinal turfgrass quality ranking data. The Dunn's test was used to separate individual means.

In year one, a significant difference was observed between the treatments for both the AUDPC ($P < 0.001$) and peak of disease data (16 February 2016) ($P < 0.001$). The lowest AUDPC was observed when $FeSO_4 \cdot 7H_2O$ was applied every two or four weeks, and at the peak of disease, $FeSO_4 \cdot 7H_2O$ applied every two weeks had the lowest disease percentage (Figure 3.1).

Interestingly, at the peak of disease, the six-week iron sulfate heptahydrate application interval had more disease than applications every eight weeks or the not treated control. A possible explanation for this observation could be that environmental conditions were ideal for the development of Microdochium patch after the effects of the previous six-week application had worn off while the timing of the eight-week application was made at a suitable moment to suppress Microdochium patch. It would be desirable to develop a reliable Microdochium patch disease model so that further research into application timing can be done in order to move away from a calendar spray program.

In year two, significant differences were observed between the treatments for both AUDPC ($P < 0.001$) and peak of disease (18 March 2017) ($P < 0.001$). The lowest AUDPC was observed when $FeSO_4 \cdot 7H_2O$ was applied every two, four, or six weeks, and at the peak of disease, the least amount of Microdochium patch was observed when $FeSO_4 \cdot 7H_2O$ was applied every two or four weeks (Figure 3.2).

In both years, turfgrass quality declined below levels acceptable for turfgrass putting greens once Microdochium patch appeared. By the month of February in both years, no application interval of $FeSO_4 \cdot 7H_2O$ was considered acceptable (Table 3.1).

In conclusion, this application interval study demonstrated that $FeSO_4 \cdot 7H_2O$ applied every two weeks suppresses Microdochium patch more consistently than a four-week application interval and more effectively than a six- or eight-week interval at the peak of disease. However, regardless of the application interval, the turfgrass quality was considered unacceptable because of either a lack of complete disease control or turfgrass thinning caused by the iron sulfate heptahydrate treatments. Future research will be necessary to determine if there is a difference between two- and three-week application intervals or if it is possible to move away from a calendar-based spray schedule by developing a weather-based application model.

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	Turf Quality 2015-2016								Turf Quality 2016-2017							
	28 Oct		16 Dec		16 Feb		1 Apr		29 Oct		23 Dec		25 Feb		6 Apr	
Every 2 wks	7.0	a[†]	5.5	a	5.4	a	5.3	a	7.0	a	6.5	ab	4.3	a	5.0	ab
Every 4 wks	6.8	a	5.4	a	5.1	ab	4.3	ab	7.0	a	7.5	a	4.8	a	5.5	a
Every 6 wks	7.0	a	4.8	a	4.0	ab	4.0	b	7.0	a	6.3	ab	4.8	a	5.1	ab
Every 8 wks	7.0	a	4.6	a	4.4	ab	4.3	ab	7.0	a	5.5	ab	4.3	a	3.5	b
NTC[‡]	6.5	a	4.3	a	3.8	b	4.3	ab	7.0	a	4.8	b	3.8	a	2.8	b

Table 3.1. Effects of 2.0 lbs. $FeSO_4 \cdot 7H_2O$ per 1,000 sq. ft. applied at different application intervals on turfgrass quality ratings in Corvallis, OR on an annual bluegrass research green. [†]Means denoted with the same letter are not significantly different according to Dunn's all-pairwise comparison test at $P \leq 0.05$. [‡]NTC is not treated control.

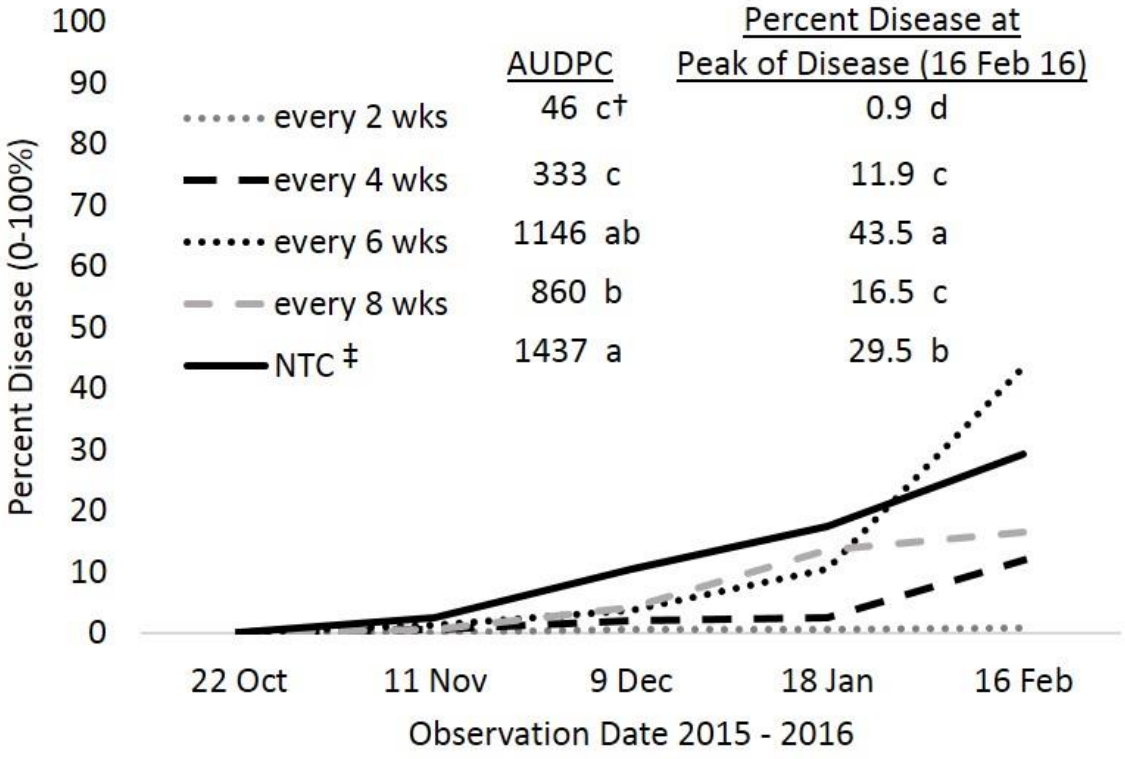

Figure 3.1. Effects of 2.0 lbs. FeSO4·7H2O per 1,000 sq. ft. applied at different application intervals on percent Microdochium patch disease progression in Corvallis, OR on an annual bluegrass research green. Area under disease progress curve (AUDPC) was quantified from the first observation date up to the peak of disease (16 Feb 2016). †Means denoted with the same letter are not significantly different according the Fisher's protected least significant difference test at $P \leq 0.05$. ‡NTC is not treated control.

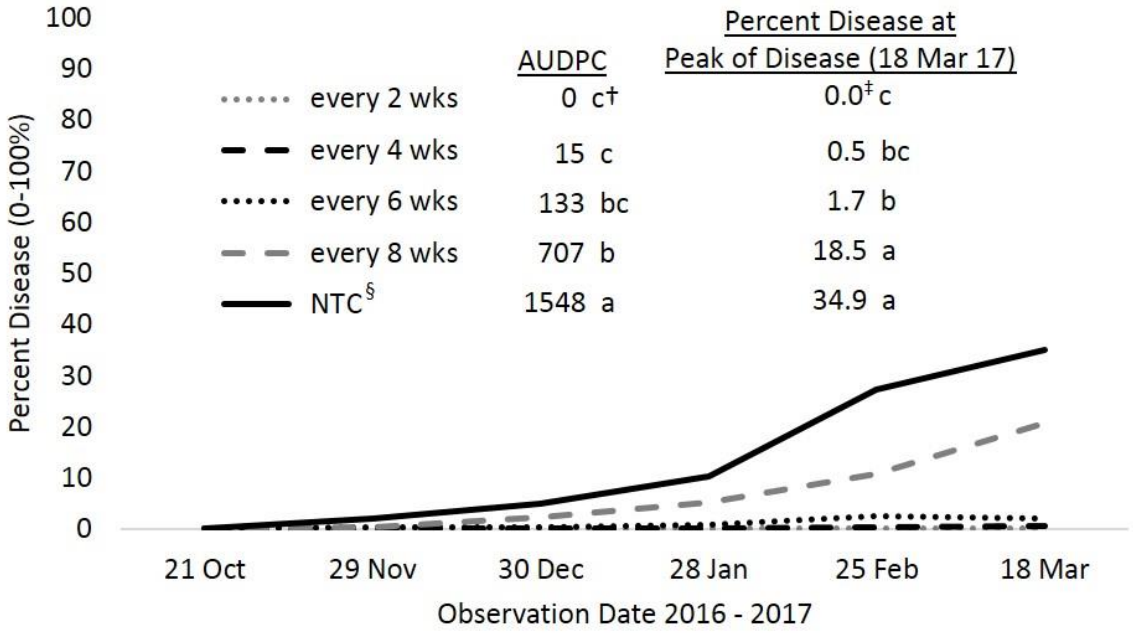

Figure 3.2. Effects of 2.0 lbs. $FeSO_4 \cdot 7H_2O$ per 1,000 sq. ft. applied at different application intervals on percent Microdochium patch disease progression in Corvallis, OR on an annual bluegrass research green. Area under disease progress curve (AUDPC) was quantified from the first observation date up to the peak of disease (18 Mar 2017 respectively). †Means denoted with the same letter are not significantly different according the Fisher's protected least significant difference test at $P \leq 0.05$. ‡Means represent back-transformed from the log means. §NTC is not treated control

Chapter 4. The effects of iron sulfate heptahydrate water carrier volumes on Microdochium patch suppression and turfgrass quality.

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Abstract

Microdochium patch is a turfgrass disease caused by the fungal pathogen *Microdochium nivale* (Fries) Samuels & I.C. Hallett that occurs most commonly in cool-humid regions such as the Pacific Northwest. Fungicide applications are the predominant method of controlling this disease, although alternatives to fungicides are desired in areas where pesticide restrictions occur. Previous research has shown that 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} applied every two weeks in 814 L ha^{-1} water carrier suppresses Microdochium patch; however, turfgrass thinning occurred. The objective of this trial was to determine if higher water carrier volumes would mitigate turfgrass thinning while still suppressing Microdochium patch. This field trial quantified the effects of four different water carrier volumes of 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} applied every two weeks on the suppression of Microdochium patch, percent green cover, and turfgrass quality of an annual bluegrass putting green in Western Oregon. This research demonstrated that 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} applied every two weeks suppresses Microdochium patch on annual bluegrass putting greens to equivalent levels regardless of water carrier volumes ranging from 1019 to 4075 L ha^{-1}. Higher percent green cover was also observed when higher water carrier volumes (3056 or 4075 L ha^{-1}) were used. While iron sulfate heptahydrate treatments suppressed Microdochium patch to less than one percent disease throughout the trial, no water carrier volume reduced annual bluegrass thinning enough to be considered acceptable for golf course putting greens.

Introduction

Microdochium patch is a turfgrass disease caused by the fungal pathogen *Microdochium nivale* (Fries) Samuels & I.C. Hallett (teleomorph *Monographella nivalis* (Schaffnit) E. Müller). In cool-humid regions, like the coastal areas of the Pacific Northwest, annual bluegrass (*Poa annua* L.) is the most common turfgrass species on putting greens, and Microdochium patch is reported to be the most important turfgrass disease in these areas (Dwyer et al., 2017; Vargas, 2005). Fungicide applications are the predominant method of controlling this disease, although alternatives to fungicides are desired in areas where pesticide restrictions occur (Ministère de l'agriculture et de la péche, 2006; Christie, 2010).

Turfgrass maintenance practices such as fertility inputs are not placed under the same restrictions as fungicides and use of fertility products that reduce disease severity may be desirable in areas where pesticide restrictions occur. Recently, applications of iron sulfate heptahydrate fertilizer have been shown to suppress both dollar spot (*Clarireedia* spp.) on creeping bentgrass (*Agrostis stolonifera* var. *palustris* (Huds.) Farw) putting greens (McCall et al., 2017), as well as Microdochium patch on annual bluegrass putting greens (Mattox et al., 2016).

In the above mentioned research, iron sulfate heptahydrate applied at 97.6 kg ha^{-1} every two weeks in a carrier volume of 814 L ha^{-1} resulted in the greatest suppression of Microdochium patch; however, these applications also resulted in turfgrass thinning and blackening of the leaves (Mattox et al., 2016). It is not yet understood why iron sulfate heptahydrate applications cause turfgrass thinning or suppress Microdochium patch. Golf course superintendents accustomed to applying rates similar to 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} report using

carrier volumes as high as 4075 L ha^{-1} (Han et al., 2017) because they hypothesize that higher carrier volumes will move the iron sulfate heptahydrate off of the leaves and reduce desiccation or blackening of the leaves (personal correspondence with golf course superintendents in Montana, Oregon, and California). It is not yet clear if these higher water carrier volumes are lessening the effects of turfgrass thinning. If the activity for disease suppression occurs in turfgrass foliage, then it could be hypothesized that a higher water carrier volume would lead to less disease suppression. The amount of water carrier typically used for fungicide applications targeting foliar diseases falls within the range of 408 L to 815 L ha^{-1} (Latin, 2011) and there is some evidence to suggest that water carrier volumes exceeding 1630 L ha^{-1} are not as effective against foliar pathogens (Couch, 1984). No research exists exploring the effectiveness of water carrier volumes greater than 815 L ha^{-1} on Microdochium patch.

This current field trial had three objectives. The first objective was to quantify the influence of different iron sulfate heptahydrate water carrier volumes on the suppression of Microdochium patch on an annual bluegrass putting green. The second objective was to determine if iron sulfate heptahydrate applications delivered using different water carrier volumes would result in differences in percent green cover. The third objective was to determine the effects of iron sulfate heptahydrate applications at different water carrier volumes on turfgrass quality.

Materials and methods

Experimental design

A field experiment was conducted at the Lewis-Brown Horticulture Farm in Corvallis, OR (latitude: 44.549668, longitude: 123.216138). The study took place on an annual bluegrass

putting green built in May 2009 using 30 cm of United States Golf Association recommended particle size sand (USGA, 2018) placed directly on the native Malabon silty clay loam (fine, mixed, mesic Pachic Ultic Argixerolls) (UC Davis, 2019) and established with annual bluegrass using sod (Bos Sod, Abbotsford, BC, Canada) that was grown from annual bluegrass plugs sourced from the Pacific Northwest. This study took place from 21 Sep 2015 to 7 Apr 2016 and was repeated on a different section of the same green from 22 Sep 2016 to 7 Apr 2017.

The study was arranged as a randomized complete block design with four replications. The experimental plots were 1.5 m^2 and the total experimental area was 30 m^2. The treatments consisted of 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} (19.6 kg Fe ha^{-1}) (Diamond Brand Dried Ferrous Sulfate Heptahydrate, Verdesian, Cary, NC) applied in four different water carrier volumes (1019, 2037, 3056, and 4075 L ha^{-1}) and a non-treated control. Applications were made every two weeks for a total of 14 applications in the first year and 13 applications in the second year (274.4 kg Fe ha^{-1} in year one and 254.8 kg Fe ha^{-1} in year two respectively). In the second year, two applications were delayed by a week because of two different weather delays, a full week of snow and rain respectively. Well water with a pH of 6.5 was used as the water carrier. The pH of the iron sulfate heptahydrate spray suspensions were 2.9, 3.0, 3.1, and 3.2 (Field Scout SoilStik, Spectrum Technologies, Aurora, IL) for the 1019, 2037, 3056, and 4075 L ha^{-1} carrier volumes respectively. Treatments were applied using a handheld boom with four XR80015 nozzles (TeeJet Technologies, Independence, OR) attached to a CO_2 pressurized backpack sprayer using a boom pressure of 280 kPa. The sprayer was calibrated to apply 1019 L ha^{-1}. Water carrier volume treatments of 1019 L ha^{-1} received the full rate of 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} applied in one spray pass; 2037 L ha^{-1} received two spray passes of a 48.8 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} (totaling 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1}); 3056 L ha^{-1} received 3 spray passes of a

32.5 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} (totaling 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1}); and 4075 L ha^{-1} water carrier volumes received four passes of a 24.4 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} (totaling 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1}). Walking speed was calibrated with a metronome and treatments were left on the foliage to dry. The trial area did not receive supplemental irrigation during the experiment.

Turfgrass maintenance

During the trial period, the green was mown once a week at 3.8 mm with clippings removed. Replicated golfer traffic representing 73 golf rounds a day was applied to the trial 5 days a week by walking over the plots with golf shoes following the protocol outlined by Hathaway and Nikolai (2005). In both years, urea was applied at 4.9 kg N ha^{-1} every two weeks throughout the trial period and in the second year, an additional 24.5 kg N ha^{-1} was applied in October to assist with recuperation from a September aerification. Total applied nitrogen during the study period in both years was 63.7 and 83.3 kg N ha^{-1}, respectively. In between trial years, irrigation was applied to replace 80% of daily evapotranspiration. The green was hand-watered to avoid drought stress when necessary. Fungicides were applied in the summer as a prophylactic treatment against Anthracnose (*Colletotrichum cereale* Manns). In the first year, flutolanil [N-[3-(1-methylethoxy)phenyl]-2-(trifluoromethyl)benzamide] was applied to suppress an outbreak of cool season brown patch (*Ceratobasidium cereale* D. Murray & L.L. Burpee) on 29 Sep 2015 and boscalid [3-pyridinecarboxamide, 2-chloro-N-(4´-chloro(1,1´-biphenyl)-2-yl)] was applied on 19 Oct 15 to suppress a dollar spot outbreak. There is little evidence that Microdochium patch is affected by applications of flutolanil (Settle et al., 2011) or boscalid (Popko et al., 2016). No other fungicides were applied to the experimental areas during the trial period for either year. In addition, sethoxydim [2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-

cyclohexen-1-one] was used to suppress creeping bentgrass in the summer periods to maintain the putting green as annual bluegrass.

Response variables

Response variables included percent disease, area under disease progress curve (AUDPC), turfgrass quality, and percent green cover using Turf Analyzer program (Karcher and Richardson, 2013; Turf Analyzer, 2018) (Figure 4.1).

Percent disease and percent green cover were quantified from two digital images per plot collected monthly using a Sony DSC-H9 camera mounted onto a battery-powered 0.31 m^2 enclosed light box with four 40-W spring lamps (TCP, Lighthouse supply, Bristol, VA). For percent disease data, a 100-point grid was overlaid onto each digital image using Sigma Scan Pro version 5.0 (Systat Software, Inc., San Jose, CA) and percent Microdochium patch was recorded using stratified sampling (Laycock and Canaway, 1980; Richardson et al., 2001). Area under disease progress curves were calculated from the beginning of the trial to the peak of disease for each year of the study using the trapezoidal method as outlined by Shaner and Finney (1977). Percent green cover was analyzed using digital image analysis (Richardson et al., 2001) with the Turf Analyzer program using the following parameters: hue: 60 to 140, saturation: 10 to 100, and brightness: 0 to 100 (Figure 4.1). The National Turfgrass Evaluation Program (NTEP) rating system was used to assign turfgrass quality ratings from one to nine with a rating of six or higher considered acceptable for golf course putting green turf (Krans and Morris, 2007).

Statistical analysis

Data were analyzed using R 3.5.2 (R Core Team, 2018) for all statistical analyses. When the data did not meet assumption of homogeneous variance, they were log transformed, which ameliorated variance distribution according to Levene's test. All transformed data were back-transformed to build figures and tables.

To answer the first objective, of whether different water carrier volumes of iron sulfate heptahydrate applications influence the suppression of Microdochium patch, AUDPC and percent disease at peak of disease were subjected to analysis of variance. To answer the second objective of whether applications of iron sulfate heptahydrate applications with higher water carrier volumes result in a higher percent green cover compared to lower water carrier volumes of iron sulfate heptahydrate, percent green cover data of the four iron sulfate heptahydrate treatments were subjected to analysis of variance (i.e. the non-treated control was excluded). Fisher's Protected Least Significant Difference (LSD) was used on AUDPC, percent disease at peak of disease, and percent green cover data to separate the means at a probability level of 0.05 when main effects were significant. To answer the third objective, the effect of these treatments on turfgrass quality, Kruskal-Wallis one-way analysis of variance was used along with the Dunn's test to separate the individual means because the data were ordinal in nature.

Results

Microdochium patch suppression

In both years, a significant difference in AUDPC and percent disease at peak of disease was observed ($P \leq 0.002$ for all four analyses). No difference in percent disease at the peak of disease or AUDPC was observed among the four water carrier volumes (Figures 4.2 and 4.3). All

iron sulfate heptahydrate treatments suppressed Microdochium patch compared to the non-treated control, although no treatments did so completely. At the peak of disease in both years, the average disease levels on plots receiving iron sulfate heptahydrate regardless of water carrier volume was less than 1% compared to 59% and 28% in the non-treated control for 2016 and 2017, respectively.

Percent green cover

Significant differences in percent green cover were observed both years during the months of December ($P < 0.001$ and 0.031, year 1 and 2 respectively), January ($P < 0.001$ and < 0.001, year 1 and 2 respectively), and February ($P < 0.001$ and 0.006, year 1 and 2 respectively), and in March and April in 2016 ($P < 0.001$ and < 0.001 respectively) (Figures 4.4 and 4.5).

On most dates when significant differences were observed, the water carrier volumes of 1019 and 2037 L ha^{-1} were in the group with the lowest percent green cover and water carrier volumes of 3056 and 4075 L ha^{-1} were in the group with the highest percent green cover. On a few dates, the 1019 L ha^{-1} water carrier volume had the lowest overall percent green cover and on one date, the water carrier volume of 4075 L ha^{-1} had a significantly higher percent green cover compared to the 3056 L ha^{-1} carrier volume (Figure 4.4). Linear regression analysis of the January data resulted in an R^2 of 0.82 in each year providing further evidence that carrier volumes of iron sulfate heptahydrate applications help to explain the differences in percent green cover observed.

Turfgrass quality

In addition to the presence of a small amount of disease (average Microdochium patch percentage was less than 1% for all iron sulfate heptahydrate treatments), turfgrass in plots receiving iron sulfate heptahydrate treatments also visually thinned out over time compared to healthy turfgrass in the non-treated control plots. Even though iron sulfate heptahydrate applied in higher water carrier volumes reduced thinning of annual bluegrass, the presence of thinning and very dark turfgrass color decreased turfgrass quality ratings. By the month of February in both years, no water carrier volume applying 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} every two weeks was considered acceptable (Table 4.1).

Discussion

Microdochium patch suppression

The observation that water carrier volumes ranging from 1019 up to 4075 L ha^{-1} did not affect the suppression of Microdochium patch by iron sulfate heptahydrate does not support the hypothesis that disease suppression would decrease as water carrier volumes increased. The lack of differences observed with the elevated water carrier volumes in this study contrast with studies that focus on fungicide applications targeting foliar pathogens like Microdochium patch. In fungicide trials, typical carrier volumes are between 408 and 815 L ha^{-1} (Latin, 2011) and there have been reports that efficacy of some contact, local penetrant, and acropetal penetrant fungicides have been lost when carrier volumes exceeded 1630 L ha^{-1} (Couch, 1984). Higher carrier volumes would potentially result in more of the iron sulfate heptahydrate to move lower in the turfgrass canopy, and putatively, less iron sulfate heptahydrate would remain on the leaves. Turfgrass roots would still be able to uptake iron from the soil, although the implications

of this regarding the suppression of Microdochium patch remain unclear until the mechanisms of disease suppression by iron sulfate heptahydrate are better understood.

One possible mechanism of disease suppression may deal with the effects of iron sulfate heptahydrate on pH. Iron sulfate applications are known to decrease soil pH (Carrow et al., 2001), and pH has been demonstrated to affect the growth of *M. nivale* (Bennett, 1933). Previous field trials have also demonstrated that acidifying products, fertilizers and sulfur fungicides can suppress Microdochium patch and lead to a lower soil pH (Brauen et al., 1975; Mattox et al., 2016) or reduce the number of fungicide applications (McDonald et al., 2018). With the effects of pH on *M. nivale* in mind, a study by Smith (1959) on annual bluegrass showed that Microdochium patch severity and soil pH in the top 12.5 mm increased as calcium carbonate applications increased. In a previous trial at Oregon State University, it was found that 97.6 kg $FeSO_4 \cdot 7H_2O$ applied every two weeks from September through April over two years resulted in a lower soil pH (pH = 5.5) compared to plots not receiving any iron sulfate heptahydrate (pH = 5.7) (Mattox, et al., 2016). It is unclear if a lower soil pH in the absence of acidifying fertilizer applications such as iron sulfate heptahydrate would suppress Microdochium patch on annual bluegrass putting greens.

It is possible that the suppression of Microdochium patch by iron sulfate heptahydrate may not occur in the soil, but in the foliage of annual bluegrass. In our trial, we used 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} and the pH of these spray suspensions were 2.9, 3.0, 3.1, and 3.2 respectively for the 1019, 2037, 3056, and 4075 L ha^{-1} carrier volumes. *In vitro* research has shown that *M. nivale* mycelium has reduced growth below pH 5.5 and that growth will not occur below pH 2.5 (Bennett, 1933). That same study showed that conidia did not germinate below pH 5.0. *M. nivale* spreads via conidia or mycelium on infected debris (Smiley et al., 2005) and conceivably, iron

sulfate heptahydrate treatments in this study may have temporarily lowered the pH of the annual bluegrass foliage to a point that the fungus could not grow fast enough to infect the annual bluegrass plants or perhaps the fungus could not grow on the foliage at all. The minor differences in spray suspension pH of the carrier volumes used in this study could explain why no differences in Microdochium patch suppression was observed among the treatments, although it is currently unknown how the pH of iron sulfate heptahydrate foliar spray applications affect the pH of the turfgrass leaves and the subsequent growth of *M. nivale*.

Other possible mechanisms for disease suppression by iron sulfate heptahydrate may be direct iron toxicity of the fungus (Forsyth, 1957) or fungal growth prevention (McCall et al., 2017). Another possibility may be a change in leaf succulence caused by osmotic effects of the iron sulfate applications (Cole, 1930) resulting in leaves that are less conducive to disease often referred to as "hardening" (Watson, 2008).

Percent green cover

Iron applications have previously been reported to reduce annual bluegrass shoot and root growth when a total of 7.6 kg Fe ha^{-1} was applied in irrigation water as iron-citrate in combination with a Hoagland's solution over the course of three weeks in a greenhouse experiment (Xu and Mancino, 2001). This may explain why iron sulfate heptahydrate applications reduced percent green cover in this current study where 19.6 kg Fe ha^{-1} was applied every two weeks (for a total of 274.4 kg Fe ha^{-1} in year one and 254.8 kg Fe ha^{-1} in year two). Another field study in Illinois observed temporary foliar dieback on Kentucky bluegrass (*Poa pratensis*) mowed at 38 mm when rates exceeded 17.7 kg Fe ha^{-1} in a single application in September applied in a 1416 L ha^{-1} water carrier (Yust et al., 1984). In that same study, the

injury from the 17.9 kg Fe ha^{-1} treatment was no longer observed 7 days after application compared to higher rates of iron (35.8 and 71.7 kg Fe ha^{-1}) where injury was still observable 7 days post application. The authors of that study concluded injury caused by iron applications was temporary and that rapid recovery occurred when environmental conditions were favorable for Kentucky bluegrass growth. In this current study on annual bluegrass, percent green cover did increase with the arrival of spring in all water carrier volumes used, although in the second year, the percent green cover did not reach pre-trial levels by the last rating date (two weeks after the last application). Even though higher versus lower carrier volumes of iron sulfate heptahydrate applications reduced the decline in annual bluegrass percent green cover, the reduction of any annual bluegrass percent green cover as a result of the iron sulfate heptahydrate applications is not desired.

An unintended outcome of this study may be to further the research of suppressing annual bluegrass with iron sulfate heptahydrate. Studies conducted on creeping bentgrass to remove annual bluegrass with iron sulfate heptahydrate applications in the spring through fall have previously been examined. A two-year study in Virginia showed that iron sulfate heptahydrate rates of 12.2, 24.4, or 48.8 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} applied every two weeks in a water carrier volume of 374 L ha^{-1} reduced annual bluegrass populations from 45% to 21% compared to a control where annual bluegrass populations decreased from 45% to 37%. (Ervin et al., 2017). In Pennsylvania, 49 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} applied every three weeks in a water carrier volume of 4075 L ha^{-1} reduced annual bluegrass when applied with only 24 kg N ha^{-1} yr^{-1} although when nitrogen inputs were increased to 147 kg N ha^{-1} yr^{-1}, iron sulfate heptahydrate applications no longer suppressed annual bluegrass (Han et al., 2017). In this current study, iron sulfate heptahydrate applications led to a reduction in annual bluegrass percent green cover by as much

as 65% in the winter months, but this appeared to be a transient effect that occurred during the coldest times of the year when annual bluegrass would not likely be growing or able to recuperate from the replicated golfer traffic that was applied. Annual bluegrass is likely at a physiological advantage over creeping bentgrass in the winter months where research has demonstrated an increase in the ratio of annual bluegrass to creeping bentgrass shoots in winter months compared to summer months (Lush, 1988; Vargas and Turgeon, 2004). It could be hypothesized that suppressing annual bluegrass in the winter would be an effective method of limiting expansion, however the recuperation of annual bluegrass percent green cover during the late winter in this trial does not provide support for that hypothesis in climates similar to the Pacific Northwest.

Turfgrass quality

Even though the average amount of disease on plots treated with iron sulfate heptahydrate was always less than 1%, turfgrass quality was considered unacceptable in both years of this study as of the February rating date because of visual observations of turfgrass thinning. The amount of thinning observed and the presence of a low amount of disease may be acceptable to some turfgrass managers where fungicide restrictions are limited or where fungicides are difficult to apply, although no study is yet to quantify the effects of the iron sulfate heptahydrate treatments on the playability of the putting green surface in spite of the thinning observed. For the majority of golf courses in the USA, fungicides are still available that will provide complete control of Microdochium patch (Vincelli et al., 2017) with only limited detriments to turfgrass health.

Conclusion

This research demonstrated that 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} applied every two weeks suppresses Microdochium patch on annual bluegrass putting greens regardless of water carrier volumes ranging from 1019 up to 4075 L ha^{-1}. A greater percent green cover was observed in both years when 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} was applied in higher water carrier volumes (3056 or 4075 L ha^{-1}) compared to lower water carrier volumes (1019 or 2037 L ha^{-1}). Turfgrass professionals managing annual bluegrass putting greens as the desired surface would be encouraged to apply iron sulfate heptahydrate at higher water carrier volumes to reduce the negative affect of iron sulfate heptahydrate applications on percent green cover. Regardless of water carrier volume, the visible thinning of the annual bluegrass sward was considered unacceptable for golf course putting greens for many months throughout the trial, reducing the likelihood that these treatments would be used in areas where fungicides are available. Further research is needed regarding the effects of these treatments on lower-value areas where some thinning may be tolerated such as on golf course tees, approaches, or fairways.

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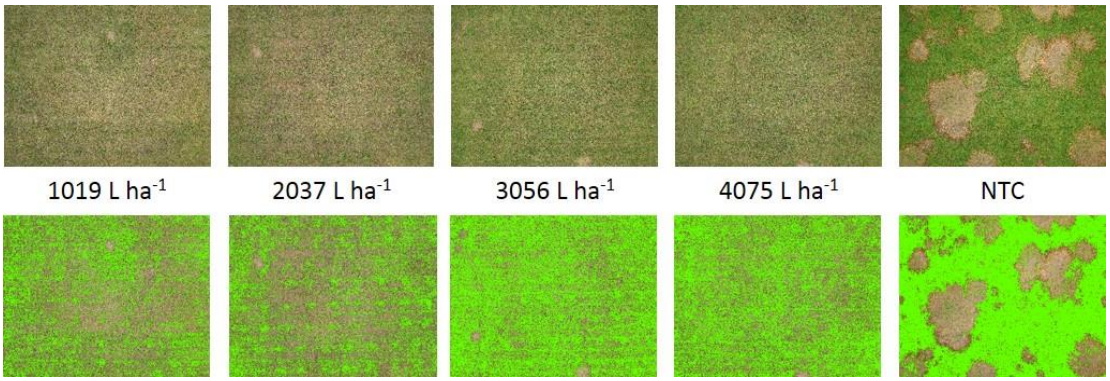

Figure 4.1. Effects of 97.6 kg FeSO4·7H2O ha-1 applied every two weeks at different water carrier volumes and non-treated control (NTC) on percent green cover on 28 Jan 18 on an annual bluegrass putting green in Corvallis, OR. Photos in the top row are of plots treated with 97.6 kg FeSO4·7H2O ha-1 every two weeks at the water carrier volume indicated. Photos in the bottom row are overlays of photos from the TurfAnalyzer software. The bright green color represents pixels that met the following parameters: hue 60 to 140, saturation 10 to 100, and brightness 0 to 100. The non-treated control photos are shown for comparison only.

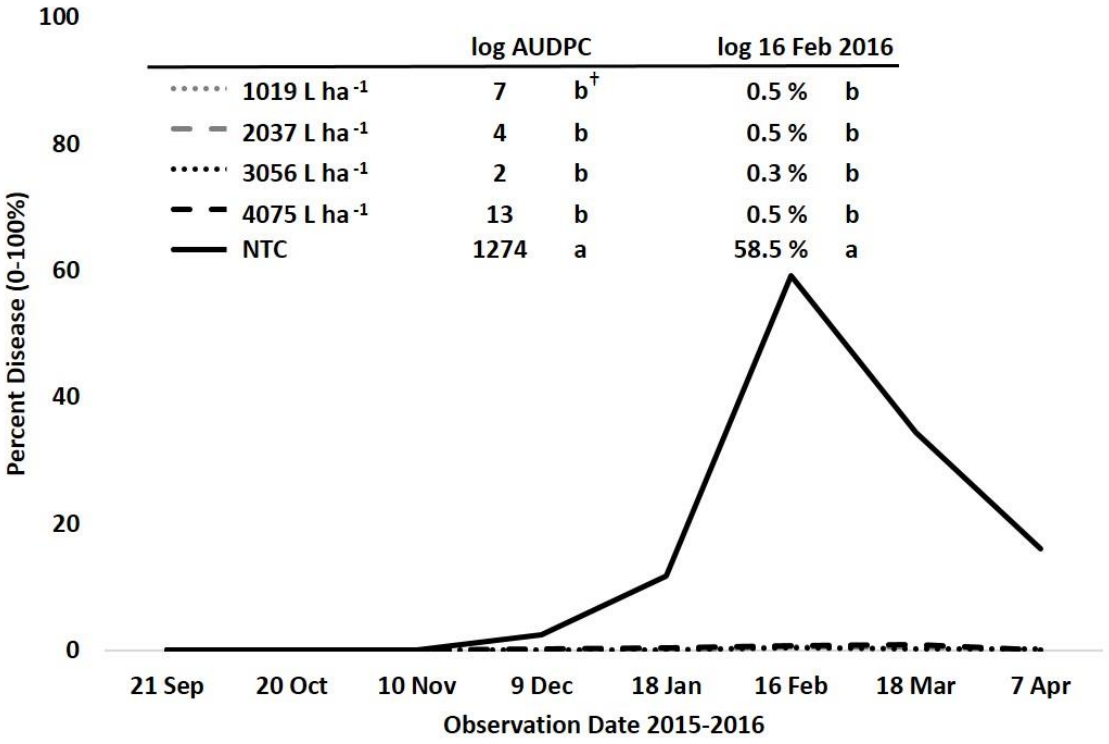

Figure 4.2. Effects of 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} applied at different water carrier volumes and non-treated control (NTC) on Microdochium patch progression on an annual bluegrass putting green in Corvallis, OR. Area under disease progress curve (AUDPC) was quantified from the first observation date up to the peak of disease (16 Feb 2016). Iron sulfate heptahydrate treatments were applied every two weeks from 21 Sep 2015 to 7 Apr 2016. † Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at $P \leq 0.05$ and means are back-transformed from the log means.

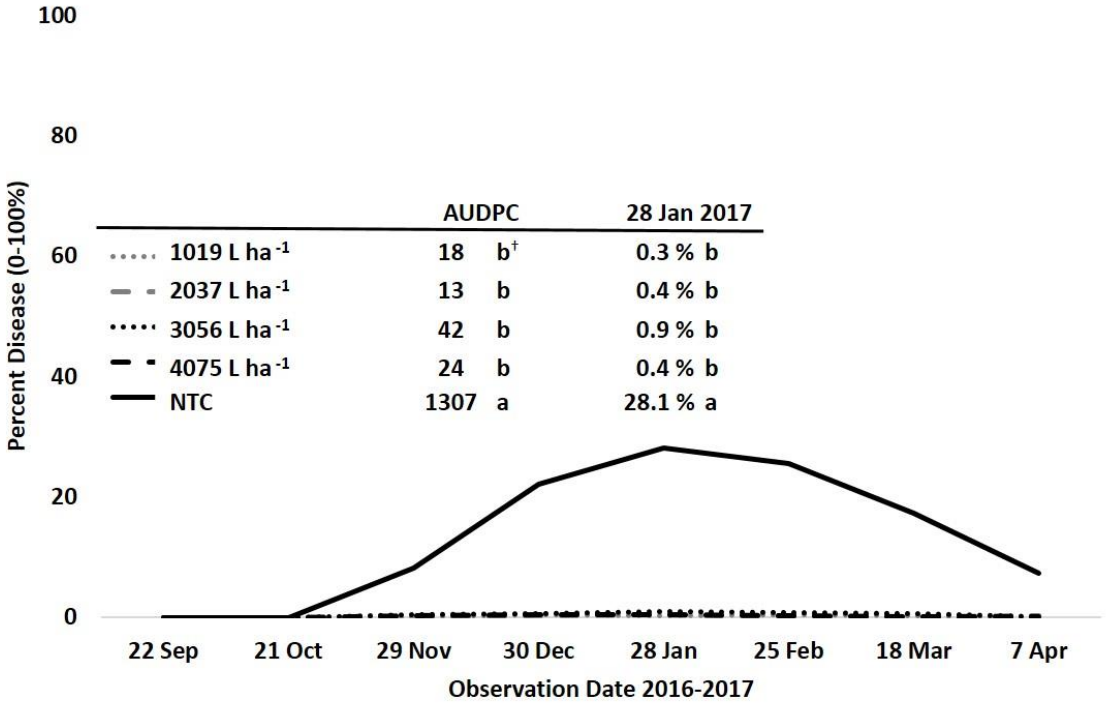

Figure 4.3. Effects of 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} applied at different water carrier volumes and non-treated control (NTC) on Microdochium patch progression on an annual bluegrass putting green in Corvallis, OR. Area under disease progress curve (AUDPC) was quantified from the first observation date up to the peak of disease (28 Jan 2017). Iron sulfate heptahydrate treatments were applied every two weeks from 22 Sep 2016 to 7 Apr 2017. † Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at $P \leq 0.05$.

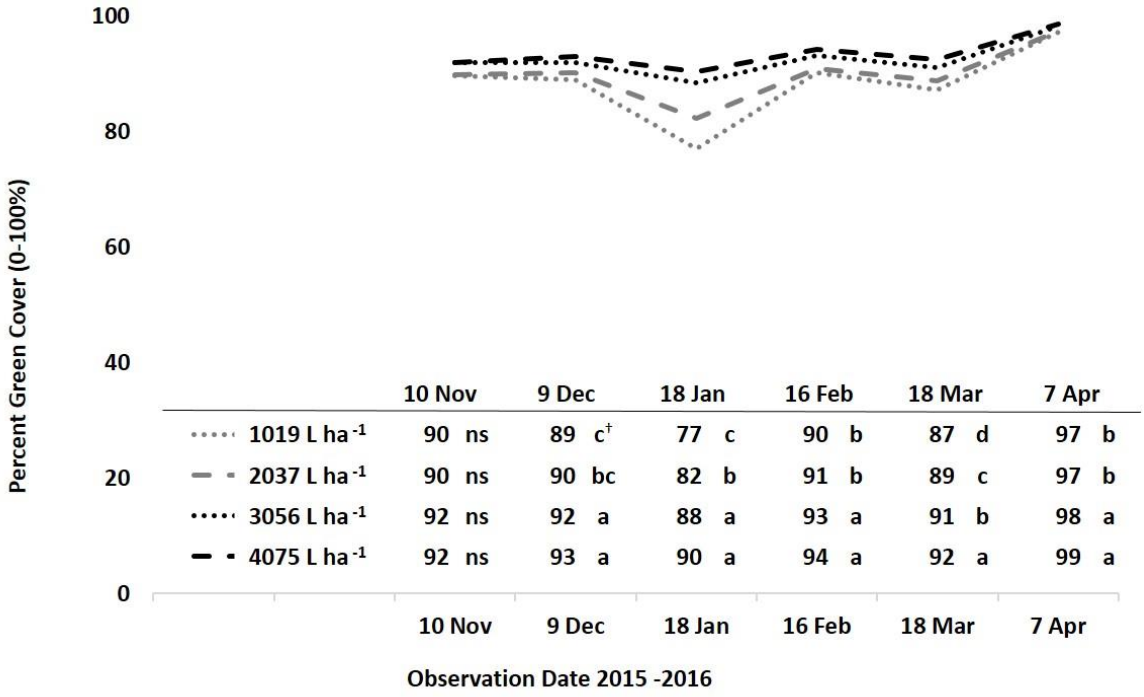

Figure 4.4. Effects of 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} applied at different water carrier volumes on percent green cover over time on an annual bluegrass putting green in Corvallis, OR. Iron sulfate heptahydrate treatments were applied every two weeks from 21 Sep 15 to 7 Apr 16. ns = not significant † Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at $P \leq 0.05$.

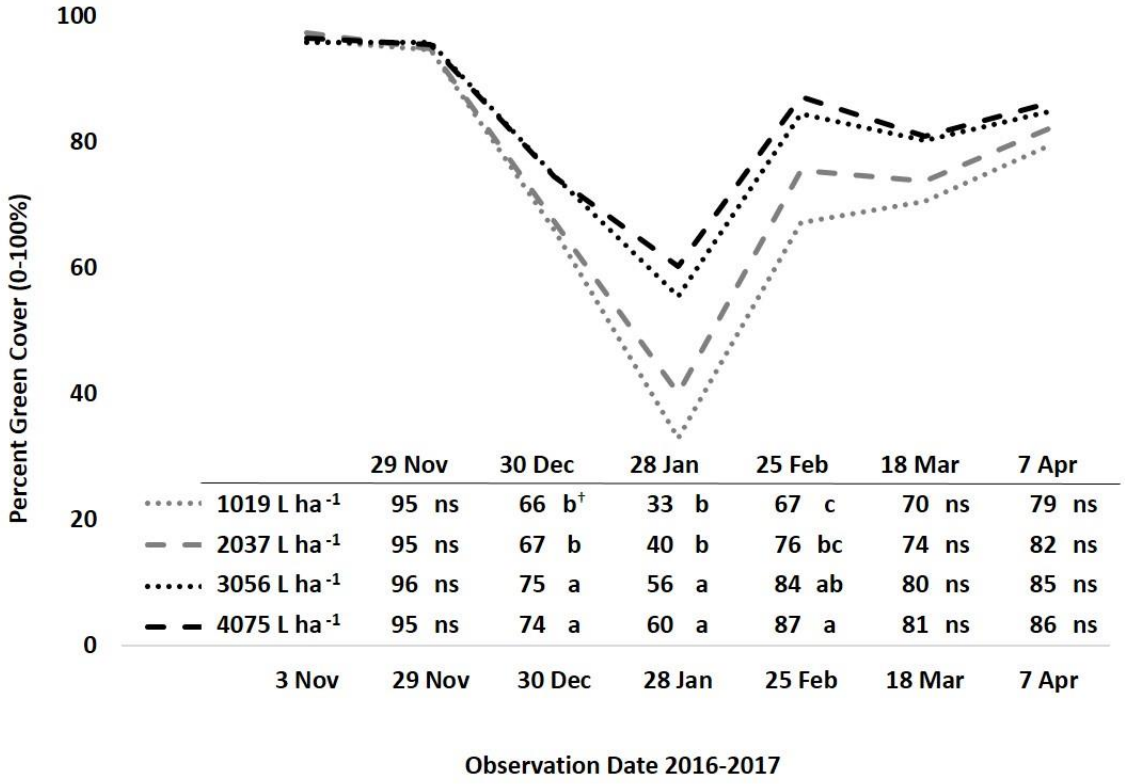

Figure 4.5. Effects of 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} applied at different water carrier volumes on percent green cover over time on an annual bluegrass putting green in Corvallis, OR. Iron sulfate heptahydrate treatments were applied every two weeks from 22 Sep 2016 to 7 Apr 2017. ns = not significant † Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at $P \leq 0.05$.

	Turf Quality 2015-2016								Turf Quality 2016-2017							
	28 Oct		16 Dec		16 Feb		1 Apr		21 Oct		23 Dec		25 Feb		7 Apr	
1019 L ha^{-1}	7.5	a†	6.5	a	5.8	a	5.9	ab	7.0	a	5.0	a	4.5	ab	4.3	a
2037 L ha^{-1}	7.5	a	6.3	a	5.6	ab	5.9	ab	7.0	a	5.0	a	4.5	ab	4.5	a
3056 L ha^{-1}	7.5	a	6.4	a	5.8	a	6.6	a	7.0	a	5.0	a	5.0	ab	4.5	a
4075 L ha^{-1}	7.5	a	6.0	a	5.5	ab	6.3	ab	7.0	a	5.0	a	5.0	a	5.0	a
Not Treated	7.0	a	5.1	a	2.8	b	3.3	b	7.0	a	4.8	a	2.8	b	4.3	a

Table 4.1. Effects of 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} applied at different water carrier volumes and non-treated control (NTC) on turfgrass quality ratings on an annual bluegrass putting green in Corvallis, OR. Iron sulfate heptahydrate treatments were applied every two weeks. †Means denoted with the same letter are not significantly different according to Dunn's all-pairwise comparison test at $P \leq 0.05$.

Chapter 5. The effects of iron sulfate and phosphorous acid on turfgrass surface pH and Microdochium patch severity on annual bluegrass

Abstract

Microdochium patch is a turfgrass disease caused by the fungal pathogen *Microdochium nivale* (Fries) Samuels and I.C. Hallett that occurs in cool-humid climates like the Pacific Northwest of North America. Iron sulfate heptahydrate applications have been shown to suppress Microdochium patch on annual bluegrass putting greens, although a loss of turfgrass quality was observed following the most effective treatments because of a loss of turfgrass density. The objective of a field experiment was to assess five rates of iron sulfate heptahydrate (0, 12, 24, 49, or 98 kg ha^{-1}) with or without 3.7 kg phosphorous acid ha^{-1} on the suppression of Microdochium patch and turfgrass quality. The field experiment found that there was no benefit in adding iron sulfate heptahydrate to phosphorous acid treatments regarding Microdochium patch suppression, however turfgrass quality was improved at lower iron sulfate heptahydrate rates when phosphorous acid was included. During the field experiment, it was noted that the spray suspensions reduced the pH of the water carrier and it was hypothesized that this reduction in pH may be the cause of the suppression of Microdochium patch. Two growth chamber experiments and two *in vitro* experiments focusing on the role that pH may play following the iron sulfate heptahydrate and phosphorous acid treatments determined that the treatments reduced the turfgrass surface pH, although this reduction in pH did not account for all of the disease suppression observed.

Introduction

Microdochium patch is a turfgrass disease caused by the fungal pathogen *Microdochium nivale* (Fries) Samuels and I.C. Hallett (teleomorph: *Monographella nivalis* (Schaffnit) E Müller) that occurs in cool-humid climates like the Pacific Northwest of North America. Disease occurs most commonly when temperature ranges are between 8 and 17°C and humidity is 90% or greater for at least 20 hours (Dwyer et al., 2017). Symptoms of Microdochium patch first appear as small patches less than 5 cm in diameter, orange brown in color, and generally do not enlarge greater than 20 cm in diameter (Smiley et al., 2005). Microdochium patch affects most cool-season grasses and is particularly severe on annual bluegrass (*Poa annua* L.) (Smiley et al., 2005) which is the most commonly reported grass grown in the Pacific Northwest on golf course putting greens (Lyman et al., 2007). The majority of golf courses apply fungicides to manage Microdochium patch although there is interest in using alternative methods to control Microdochium patch because of pesticide restrictions (Ministère de l'agriculture et de la péche, 2006; Christie, 2010), documented fungicide resistance (Allan-Perkins et al., 2019), and as a part of an integrated pest management strategy (San Francisco, 2019).

Research has demonstrated that iron sulfate heptahydrate applications suppress Microdochium patch on annual bluegrass putting greens, although the most effective rates for disease suppression (97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1}) led to unacceptable turfgrass quality because of turfgrass thinning (Mattox et al., 2016). Experiments focusing on comparing different application frequencies of 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} on the suppression of Microdochium patch and turfgrass quality showed that frequencies greater than every four weeks did not consistently suppress Microdochium patch or improve turfgrass quality compared to the non-treated control. These experiments also demonstrated that applications every 2 weeks was necessary to suppress

Microdochium patch to less than 1% in each year (Mattox et al., 2019 – also chapter 3 of this dissertation). Another experiment focusing on water carrier volumes of 97.6 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} applied every two weeks demonstrated that 3056 and 4075 L ha^{-1} spray carrier volumes resulted in a higher green cover percentage compared to 1019 or 2037 L ha^{-1}, however even at these higher volumes, turfgrass quality was not considered acceptable for golf course putting greens (Mattox et al., 2019 – also chapter 4 of this dissertation).

Suppression of Microdochium patch on annual bluegrass by phosphorous acid has previously been documented (Dempsey, 2012; Mattox et al., 2020). There is evidence that phosphorous acid inhibits oomycete water molds (Guest and Grant, 1991) and growth of *M. nivale in vitro* has also been suppressed by phosphorous acid applications, demonstrating a fungistatic effect on mycelial expansion and a reduction of conidial germination (Dempsey, 2016). Phosphorous acid is listed as a product that induces host plant resistance (Fungicide Resistance Action Committee, 2019) and phytoalexin production has been found to increase in annual bluegrass and creeping bentgrass plants following phosphorous acid applications (Dempsey, 2016). When phosphorous acid (3.7 kg H_3PO_3 ha^{-1}) was applied every two weeks in combination with sulfur applications (12.2 kg S ha^{-1}) suppression of Microdochium patch was improved compared to applying either alone (Mattox et al., 2020). It is unclear why there was an added benefit from the sulfur and phosphorous acid combination, however it could be postulated that the sulfur applications may be lowering the pH to create an environment less conducive to the growth of *M. nivale* (Bennett, 1933) while phosphorous acid is inducing defense mechanisms in the plant and / or having a fungistatic effect on *M. nivale* ((Fenn and Coffey, 1984; Grant et al., 1990; Dempsey, 2016).

Suppression of Microdochium patch by iron sulfate heptahydrate has previously been documented (Mattox et al., 2016, chapters 3 and 4 of this dissertation), although there is only limited research into the mechanisms behind plant disease suppression with iron. Direct iron toxicity has been speculated to reduce rust (*Puccinia sp.*) in wheat (Forsyth, 1957) and has been shown to suppress the growth of *Clarireedia spp*. *in vitro* (McCall et al., 2017). A change in leaf succulence caused by osmotic effects has also been proposed (Cole, 1930) that may lead to "hardening" (Watson, 2008) of the leaves that are less conducive to infection. In the course of the current field study, it was observed that the pH of the spray suspensions decreased from approximately 6.7 to as low as 2.2 when iron sulfate heptahydrate or phosphorous acid was added to the well water for the field plot applications. Since previous studies have shown that *M. nivale* will not grow *in vitro* below a pH of 2.5 (Bennett, 1933), it was hypothesized that the spray suspensions may be altering the pH of the leaf environment causing unfavorable conditions for *M. nivale*. Previous studies have found that *M. nivale* does not grow *in vitro* at a pH below 2.5 and that growth is reduced below a pH of 5.5 (Bennett, 1933). Another study in Western Oregon found that multiple applications of iron sulfate heptahydrate decreases soil pH (Mattox et al., 2016) suspected to be caused by the reaction of iron sulfate with water to produce hydrogen ions (Carrow et al., 2001). Maintaining soil pH below 5.5 in order to influence *M. nivale* would be ill advised because of the increased risk of Al or Mn toxicity (Foy, 1992; Liu, 2001) as well as a reduction in nutrient availability (Carrow et al., 2001).

It could be hypothesized that reduced rates of iron sulfate heptahydrate applied in combination with phosphorous acid may provide a similar added benefit as the aforementioned sulfur and phosphorous acid combination regarding Microdochium patch suppression, permitting lower application rates of iron sulfate heptahydrate to suppress disease while maintaining a

higher turfgrass quality compared to when higher rates of iron sulfate heptahydrate are applied alone. To answer this hypothesis, a field experiment was conducted over two years in Western Oregon to compare 5 rates of iron sulfate heptahydrate with or without phosphorous acid on the suppression of Microdochium patch and turfgrass quality.

During the course of the field experiment, it was anecdotally observed that both iron sulfate heptahydrate and phosphorous acid reduced the pH of the water carrier suspensions to as low as pH 2.2 compared to the well water used to apply these products (having a pH ranging from approximately 6.7 to 7.0). It was hypothesized that foliar spray applications of iron sulfate heptahydrate and/or phosphorous acid every two weeks may be decreasing the turfgrass surface pH, possibly leading to an unfavorable environment for *M. nivale*, thus suppressing Microdochium patch. For this reason, two additional experiments took place in a controlled environment (using a growth chamber) to explore if and for how long the spray suspensions may be affecting the pH of the leaf surface and to explore the effects of an acidifying agent on the suppression of Microdochium patch on annual bluegrass. Based on the results of the growth chamber experiment, an additional *in vitro* study took place focusing on elucidating the effects of iron sulfate heptahydrate and pH on *M. nivale* in pure culture.

Materials and methods

Factorial field experiment of five rates of iron sulfate heptahydrate applied with or without phosphorous acid

A field experiment took place at the Lewis-Brown Horticulture farm in Corvallis, OR (latitude: 44.549668, longitude: 123.216138) on an annual bluegrass putting green established in 2009 from sod that was grown from annual bluegrass sourced in the Pacific Northwest (Bos Sod, Abbotsford, BC, Canada). The sod was placed on 30 cm of United States Golf Association

recommended particle size sand (USGA, 2018) located on a native Malabon silty clay loam (fine, mixed, mesic Pachic Ultic Argixerolls) (UC Davis, 2019). The experiment took place from 29 September 2016 to 30 April 2017 and was repeated on a different section of the same green from 28 September 2017 to 30 April 2018.

The study was arranged as a five by two factorial randomized complete block design with four replications. The treatments consisted of five rates of iron sulfate heptahydrate (Diamond Brand Dried Ferrous Sulfate Heptahydrate, Verdesian, Cary, NC): 0, 12, 24, 49, or 98 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} and two rates of phosphorous acid (Duraphite 12, Boise, ID): 0 or 3.7 kg H_3PO_3 ha^{-1}. The experimental plots were 1.5 m^2 with a total experimental area of 60 m^2. Treatments were applied using a CO_2 pressurized backpack sprayer and a handheld spray boom equipped with four XR80015 nozzles (TeeJet Technologies, Independent, OR) using a boom pressure of 280 kPa and a spray carrier volume of 814 L ha^{-1}. Spray boom displacement was calibrated using a metronome and treatments were left on the foliage to dry.

The green was mown at 3.8 mm once a week with clippings removed. Traffic replication of 73 rounds a day occurred five days a week by walking over the plots with golf shoes following the protocol as outlined by Hathaway and Nikolai (2005). During the trial period, nitrogen was applied at a rate of 4.9 kg N ha^{-1} every two weeks as urea. In both years, additional nitrogen was applied to assist with recuperation from a September aerification for a total of 34.4 kg N ha^{-1} applied in September and October 2016 and 41.65 kg N ha^{-1} applied in September and October 2017. In total, 88.2 kg N ha^{-1} and 85.8 kg N ha^{-1} were applied during the trial periods in each year respectively. No irrigation was applied during the trial and 80% evapotranspiration replacement was applied in between trial years. In the summer, hand-watering was used to avoid drought stress and fungicides were used as a prophylactic against anthracnose. No fungicides

were applied to the trial during the experimental period. In order to control any bentgrass infestation, the herbicide sethoxydim [2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one] (Segment, BASF, Ludwigshafen, Germany) was applied prior to and in between trial years.

Response variables included Microdochium patch percentage, area under disease progress curve, and turfgrass quality. Because of visible turfgrass thinning and darkening of the turfgrass leaves by the treatments, digital image analysis was not practical to quantify disease percentage, therefore Microdochium patch percentage was quantified using stratified sampling (Laycock and Canaway, 1980; Richardson et al., 2001) by overlaying a 100-point digital grid with Sigma Scan Pro version 5.0 (Systat Software, Inc., San Jose, CA) onto two digital images collected per plot. Images were collected in the same location each time using a battery-powered 0.31 m^2 enclosed light box equipped with four 40-W spring lamps (TCP, Lighthouse supply, Bristol, VA) with a Sony DSC-H9 camera. Monthly Microdochium patch percentage data were used to build area under disease progress curves by using the trapezoidal method (Shaner and Finney, 1977). Turfgrass quality ratings were assigned using the National Turfgrass Evaluation Program rating system from one to nine with a rating of six or greater considered to be acceptable (Krans and Morris, 2007).

The disease percentage data at the peak of disease in both years satisfied the assumptions of analysis of variance although the AUDPC data did not meet the assumption of homogeneous variance as measured by the Brown-Forsythe test in either year ($P < .0001$ in both years). A cuberoot transformation ameliorated the AUDPC data to meet this assumption ($P = 0.240$ in year one and $P = 0.068$ in year two). The disease percentage data and the cuberoot transformed AUDPC data were analyzed using a factorial design analysis in R (R Core Team, 2018) and

Tukey's honest significant difference was used to assess pairwise comparisons (Tukey, 1949). The cuberoot transformed AUDPC data were back transformed when necessary to construct means tables. The ordinal turfgrass quality data (Karcher, 2000) were analyzed using the Kruskal-Wallis (Kruskal and Wallis, 1952) and pairwise comparisons were assessed using Dunn's test (Dunn, 1964) in R.

Effects of iron sulfate heptahydrate and phosphorous acid on the turfgrass surface pH

A growth chamber study focusing on treatment effects of the turfgrass surface pH was set-up in an identical manner as the field experiment using the same five rates of iron sulfate heptahydrate and two rates of phosphorous acid in a factorial randomized complete block design with four replications. The experimental units consisted of pots that were prepared by removing a 108 mm diameter plug of annual bluegrass 92 mm in depth using a golf course cup cutter (Par Aid HIO, Lino Lakes, MN) and placing the plug into a 108 mm interior diameter polyvinyl chloride coupler that was 92 mm in depth. The plugs were collected from four different designated blocks on the same putting green on 12 April 2019 and 13 May 2019 for each run respectively. Any excess space on the underside of the pot was filled with the same particle sized sand as the putting green. The bottom of the pot was covered with a piece of geo-textile cloth and attached using a rubber band. The pot was then placed in a 1.42-liter plastic container with a 140 mm base diameter, 89 mm in height and immediately placed in an on-site cold room set at 4°C. Within 18 hours, the pots were removed from the cold-room, one pot from each block was randomly assigned a treatment and placed accordingly in the middle of a 1 m^2 area that was outlined on the ground. Treatments were applied to the surface of the turfgrass pots using the same sprayer set up as the field experiment described above. Immediately after spraying, the

plugs were taken to the Oregon State University campus (approximately a 10-minute drive) and placed in a growth chamber (Percival LT-36VL, Percival-Scientific, Perry, IA) set at a constant 4°C with a 10 hour daylength and an average light intensity of 42 μmol m^{-2} s^{-1}.

The pots were placed in the growth chamber using a completely randomized design and were re-randomly assigned a location in the growth chamber after each pH reading (every 1 or 2 days). The turfgrass surface pH was quantified using a flat electrode FieldScout SoilStik pH meter (Spectrum Technologies, Aurora, IL) that was placed on top of the turfgrass pot using a support specifically built for the purpose. The pH meter was calibrated using pH standards of 4.0 and 7.0 before assessing pH and after every 15 readings. One milliliter of deionized water was placed on the surface of the turfgrass leaves and the pH meter was placed directly on top of the deionized water. A standard weight (555 grams) was placed on top of the pH meter using the support in order to apply a consistent downward pressure onto the meter and to maintain uniform readings among the pots. Once the water infiltrated into the turfgrass canopy, a timer was set to two minutes in order to allow time for the measurement to stabilize and the pH was recorded at two minutes. A permanent marker was used to make a mark along the edge of the pot in order to indicate where the reading was made so as to avoid testing the same area twice. The pot was then placed in the newly randomly assigned location in the growth chamber, the pH meter was rinsed with deionized water and then dried using a chem-wipe and this process was repeated for every reading. Turfgrass surface pH data were analyzed in a repeated measures analysis of variance, standard t-tests were used to make multiple comparisons and family-wise errors were corrected using the Holm's method (Holm, 1979) using Proc Mixed in SAS 9.4 (SAS Institute Inc., Cary, N.C).

Effects of spray suspension pH and turfgrass surface pH on Microdochium patch severity

A second growth chamber experiment focusing on the effects of spray suspension pH on the pH of the leaf surface and Microdochium patch severity took place. Turfgrass pots were prepared identically as described above for the turfgrass surface pH assessment experiment and placed in identical plastic containers. The annual bluegrass was collected on 9 September 2019 and 12 September 2019 for each experimental run respectively. Treatments included 0.05%, 0.125%, and 0.5% H_2SO_4 solution prepared by diluting a 5% v/v H_2SO_4 solution (AquaSolutions, Inc., Deer Park, TX) with well water, 98 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} (Diamond Brand Dried Ferrous Sulfate Heptahydrate, Verdesian, Cary, NC), 3.7 kg H_3PO_3 ha^{-1} (Duraphite 12, Boise, ID), the combination of 98 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} and 3.7 kg H_3PO_3 ha^{-1}, and a well water control. Treatments were applied to the surface of the turfgrass pots using the same sprayer set up as the field experiment described above. Immediately after spraying, the plugs were taken to the Oregon State University campus and placed in a growth chamber set to 1.5 C nighttime temperature and 10.5 C daytime temperature with the daylength of 10 hours, and the same light intensity as above in order to encourage Microdochium patch growth. This daylength and temperature corresponds closely to the weather conditions in Oregon during February when Microdochium patch is prevalent. In contrast with the previous experiment, six hours after the pots were sprayed, the turfgrass surface pH was assessed, and two 10 mm diameter plugs of *M. nivale* were applied next to each other mycelium side down approximately 25 mm from the edge of one side of the pot. The *M. nivale* sample used in this study was collected in 2017 from annual bluegrass collected from the same putting green as the annual bluegrass used in the field and growth chamber experiment. The identity of the fungus was determined by the Center for Genome Research and Biocomputing at Oregon State University using the ITS1F and ITS4

primers (White et al., 1990) and matching the results with known samples using the Basic Local Alignment Search Tool in Genbank (National Institute of Health, Bethesda, MD). For both runs of the trial, *M. nivale* was collected from Petri dishes where it was growing in pure culture on one quarter strength potato dextrose agar from 15 to 18 days prior to inoculation. After the plugs were placed on the pots, the pots were immediately covered with an identical plastic container as the base container and the seam between the plastic containers was sealed using plastic wrap in order to increase the humidity in the plastic containers. Within 24 hours, water accumulated on the sides of the plastic containers indicating that the humidity was near 100%. Every seven days for three weeks, the top plastic container was removed, the pH was tested as described above, the location tested was marked on the side of the pot with a marker in order to avoid sampling the same area in the future, and a photograph was taken of the surface of the turfgrass sward using a camera stand and the rear-facing Apple iPhone 4 camera (Apple Inc., Cupertino, CA). A ruler was placed on the edge of the turfgrass sward in each picture for scale. Upon collection of the photo, 50 ml of tap water was added to the side of the containers using a graduated cylinder without wetting the leaves in order to ensure a high level of humidity inside the containers. The containers were again sealed with fresh plastic wrap and placed in a newly randomized location in the growth chamber. The annual bluegrass did visually grow approximately 12 mm over the course of the 21-day experiment, however the blades were not trimmed out of precaution that this would affect the area affected by Microdochium patch or the turfgrass surface pH readings.

Response variables were turfgrass surface pH and Microdochium patch severity quantified as mm^2 of area affected. Turfgrass surface pH was collected every 7 days using a SoilStik pH meter and Microdochium patch severity was quantified by measuring the area of the turfgrass affected by Microdochium patch in a digital image by using the trace tool in image J

(National Institute of Health, Bethesda, MD) with a ruler used as a scale of reference in each image. Turfgrass surface pH data were analyzed in a repeated measures analysis of variance, standard t-tests were used to make multiple comparisons, and family-wise errors were corrected using the Holm's method (Holm, 1979) using Proc Mixed in SAS 9.4 (SAS Institute Inc., Cary, N.C). Microdochium patch area data in run one did not meet the assumption of homogeneous variance (Brown-Forsythe P-value of 0.012), therefore these data were modified using a cuberoot transformation, resulting in a Brown-Forsythe P-value of 0.128. A significant difference was found in the run one data ($P < 0.001$) and Tukey's honest significant difference was used to assess pairwise comparisons. Means for run one data were back transformed for the construction of means tables. In run two, Microdochium patch severity data did not meet the assumption of homogeneous variance (Brown-Forsythe P-value = 0.018). Attempts at transforming the data were unsuccessful, therefore the non-parametric Kruskal-wallis analysis was used and pairwise comparisons were assessed using Dunn's test. SAS 9.4 was used for all statistical analysis with the exception of the Brown-Forsythe, Kruskal-wallis, and Dunn's test, in these cases R was used.

The effects of iron sulfate heptahydrate, chelated iron, and magnesium sulfate heptahydrate on *Microdochium nivale in vitro*

An *in vitro* study took place to elucidate possible mechanisms of *M. nivale* suppression by iron sulfate heptahydrate. The *M. nivale* sample used in the *in vitro* studies was derived from the same source as was used in the growth chamber study above. The sample was grown in pure culture on quarter strength potato dextrose agar prepared using 6 grams of potato dextrose broth (Hi-Media, Mumba, India) and 12.5 grams of Bacto Agar (Difco Laboratories, Franklin Lakes, NJ) per 1000 ml of deionized water. Using sterilized deionized water, a 2,000 ppm Fe concentration was prepared using iron (II) sulfate heptahydrate (Sigmapharm Laboratories,

Bensalem, PA), the same Fe concentration was prepared using Sprint 330 sodium hydrogen ferric DTPA [$C_{14}H_{22}FeN_3NaO_{10}$] (BASF, Research Triangle Park, NC), and the equivalent concentration of sulfate in the iron sulfate heptahydrate suspension was prepared using magnesium sulfate heptahydrate (Sigmapharm Laboratories, Bensalem, PA). Mixtures of 10 ppm, 100 ppm, and 500 ppm of iron as well as the corresponding sulfate equivalent to iron sulfate heptahydrate were each prepared in a 250 ml beaker by adding the nutrient suspensions to sterilized quarter strength potato dextrose agar allowed to cool to 45°C. All beakers as well as pH standards were placed in a Precision water bath model 183 (Thermo Scientific, Marietta, OH) set to provide a temperature of 45°C inside of a laboratory hood. Once the potato dextrose agar was 45°C, a FieldScout SoilStik pH meter (Spectrum Technologies, Aurora, IL) was calibrated using standards of pH 4.0 and 7.0 at 45°C and the mixtures for the suspensions (with the exception of the 500 ppm Fe as iron sulfate heptahydrate because preliminary trials demonstrated that the turfgrass surface pH + 24 hours was approximately 4.0) were modified to pH 4.0 using the same pH meter. The mixtures required decreasing the pH to 4.0 using a 1 molar solution of L-Lactic acid (ThermoFisher Scientific, Ward Hill, MA) except for 500 ppm Fe as DTPA ferrous chelate, which required the pH to be increased to pH 4.0 using a 1 molar solution of sodium hydroxide (ThermoFisher Scientific, Ward Hill, MA). Four plates of each mixture were prepared by adding 20 ml of each mixture to a sterile Petri dish (VWR, Radnor, PA). The Petri dishes were left overnight in a laboratory hood to in order to gel and to bring them to room temperature. The following morning, a 10 mm plug of *M. nivale* was placed mycelium side down in the center of three of the four Petri dishes for each mixture and sealed with parafilm (Bemis Flexible Packaging, Neenah, WI). The fourth Petri dish was used to verify the pH of the surface of the media at the start of the trial and at the conclusion of the experiment and was sealed with

parafilm after the pH was recorded. The turfgrass surface pH was quantified using the same flat-tip electrode pH meter as was used to modify the pH of the mixtures by placing the pH meter on top of the plate using a support constructed for that purpose. Once the meter was in place, a timer was set to 2 minutes in order to allow the pH meter to stabilize and the pH was recorded. All the plates were placed in a cardboard box with a lid in order to keep light from entering and stored at room temperature (approximately 22°C). After 14 days, the plates were photographed, and the pH of the fourth Petri dish was recorded following the same protocol.

The response variable was area of *M. nivale* expansion quantified using the trace tool in image J (National Institute of Health, Bethesda, MD) with a ruler used as a scale of reference in each image. The area of *M. nivale* expansion data satisfied the assumptions of homogeneous variance with a Brown-Forsythe P-value of 0.772 for run 1 and a P-value of 0.542 for run 2. A significant difference was found for both runs ($P < 0.001$ for both runs), therefore Tukey's honest significant difference was used to assess pairwise comparisons for the construction of means tables.

<u>The effects of pH amended media using iron sulfate heptahydrate and lactic acid on *Microdochium nivale*</u>

Six concentrations of iron sulfate heptahydrate (10 ppm, 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm of iron), six concentrations of one molar lactic acid chosen to encompass the range of pH observed from the iron sulfate heptahydrate suspensions (500 ppm, 999 ppm, 4975 ppm, 9898 ppm, 14778 ppm, and 19608 ppm) and a non-amended control were prepared as described above using quarter strength potato dextrose agar. The pH of these plates was not further modified and six plates of 20 ml of each mixture were prepared and placed overnight in a laboratory hood. The following morning, a 10 mm plug of *M. nivale* was placed mycelium side

down in the center of three of the six plates and sealed with parafilm. The pH of the surface of the remaining three plates was recorded using a SoilStik pH meter as described above and these plates were also sealed with parafilm. All of the plates were placed in a cardboard box with a lid to prevent light from entering and stored at room temperature. At 3 days, 6 days, and 10 days post inoculation, the three plates of each treatment with *M. nivale* were photographed and the turfgrass surface pH of the remaining three plates was recorded and the plates were immediately re-sealed with parafilm.

The response variables were turfgrass surface pH of the growth media and area of *M. nivale* expansion quantified using the trace tool in image J (National Institute of Health, Bethesda, MD) with a ruler used as a scale of reference in each image. The area of *M. nivale* expansion data satisfied the assumptions of homogeneous variance with a Brown-Forsythe P-value of 0.455 for run 1 and a P-value of 0.107 for run 2. A significant difference was found for both runs ($P < 0.001$ for both runs), therefore Tukey's honest significant difference was used to assess pairwise comparisons for the construction of means tables.

Results

Factorial field experiment of five rates of iron sulfate heptahydrate applied with or without phosphorous acid

A significant iron by phosphorous acid interaction was observed in both years for Microdochium patch percentage at the peak of disease ($P < 0.001$ in both years) (Figures 5.1 and 5.2). All treatments suppressed Microdochium patch compared to the non-treated control at the peak of disease in both years. All iron sulfate heptahydrate rates applied in combination with phosphorous acid and the 49 and 98 kg $FeSO_4 \bullet 7H_2O$ ha^{-1} applied in the absence of phosphorous acid were in the group that provided the greatest suppression of Microdochium patch. Because

phosphorous acid applied alone was already in the group with the lowest Microdochium patch severity, adding iron sulfate to phosphorous acid applications did not improve Microdochium patch suppression.

Regarding the AUDPC data, in the first year, there was a main effect difference for the phosphorous acid and iron sulfate heptahydrate factors ($P < 0.001$ for both factors). Phosphorous acid suppressed Microdochium patch compared to treatments not receiving phosphorous acid. Iron sulfate heptahydrate rates of 24, 49, and 98 kg $FeSO_4 \bullet 7H_2O$ ha^{-1} suppressed Microdochium patch compared to no iron sulfate heptahydrate and the 98 kg $FeSO_4 \bullet 7H_2O$ ha^{-1} yielded the greatest disease suppression. In year two, there was a significant interaction ($P < 0.001$) and all treatments suppressed Microdochium patch compared to the non-treated control with the exception of 12 kg $FeSO_4 \bullet 7H_2O$ ha^{-1} applied every two weeks.

In neither year did a treatment result in a turfgrass quality rating considered acceptable for golf course putting greens throughout every month of the trial (Tables 5.1 and 5.2). This was because of either the presence of Microdochium patch, unacceptable turfgrass color resulting from a blackening of the turfgrass leaves or thinning of the turfgrass stand. When a separation of means was found to be significant, the 98 kg $FeSO_4 \bullet 7H_2O$ ha^{-1} rate with or without phosphorous acid was in the lowest group of turfgrass quality ratings because of visible thinning of the turfgrass stand in spite of the low percentages of Microdochium patch observed. The treatments that ranked in the group with the highest turfgrass quality rating were most frequently 24 or 49 kg $FeSO_4 \bullet 7H_2O$ ha^{-1} applied in combination with phosphorous acid because these applications suppressed Microdochium patch while resulting in less pronounced turfgrass thinning compared to higher iron sulfate heptahydrate rates.

Effects of iron sulfate heptahydrate and phosphorous acid on the turfgrass surface pH

The repeated measures analysis indicated a significant interaction between iron sulfate heptahydrate treatments and day in run one ($P = 0.022$) and between phosphorous acid treatments and day in both runs ($P < 0.001$ in run one and $P = 0.002$ in run two). In both runs of the experiment, the well water control was always in the group with the highest turfgrass surface pH and was with only a few exceptions significantly different from all other treatments on all rating dates (Tables 5.3 and 5.4). When differences existed among the remaining treatments, the 12 kg $FeSO_4 \bullet 7H_2O$ ha^{-1} rate and the 3.8 kg H_3PO_3 ha^{-1} rate frequently resulted in a higher turfgrass surface pH compared to 98 kg $FeSO_4 \bullet 7H_2O$ ha^{-1} applied alone or any combination of iron sulfate heptahydrate and phosphorous acid.

Effects of spray suspension pH and turfgrass surface pH on Microdochium patch

The repeated measures analysis indicated a significant interaction between treatments and day in both runs ($P < 0.001$). The well water control was always in the group with the highest turfgrass surface pH (Table 5.5) in both runs of the experiment. Treatments that included 98 kg $FeSO_4 \bullet 7H_2O$ ha^{-1} with or without phosphorous acid had a significantly lower turfgrass surface pH compared to the well water control 6 hours and 7 days after the treatments were applied. The highest rate of sulfuric acid (0.5% 1 Molar H_2SO_4) had a lower turfgrass surface pH compared to the well water control 6 hours, seven, and fourteen days after the treatments were applied. Microdochium patch was visible on half of the experimental units after 14 days in each run of the experiment. After 21 days, Microdochium patch symptoms further expanded and the final data collection for each run was completed. Regarding Microdochium patch 21 days post inoculation, a significant treatment effect was observed for both runs ($P <0.001$ for both runs). In

both runs, only pots receiving 98 kg $FeSO_4 \bullet 7H_2O$ ha^{-1} with or without phosphorous acid suppressed Microdochium patch compared to the non-treated control regardless of the inclusion of phosphorous acid in the treatments.

The effects of iron sulfate heptahydrate, chelated iron, and magnesium sulfate heptahydrate on *Microdochium nivale in vitro*

Importantly, the pH of the media of the witness plate decreased 15 days post-preparation (14 days post addition of *M. nivale*) from approximately 4.0 to 3.6 for media amended with 100 ppm $FeSO_4 \bullet 7H_2O$ in both runs and to 3.4 and 3.5 for media amended with 500 ppm $FeSO_4 \bullet 7H_2O$ in runs 1 and 2 respectively (Table 5.6 and Figure 5.3). The observation that the pH of the media amended with these $FeSO_4 \bullet 7H_2O$ concentrations leads to a confounding factor and limits how the results can be interpreted. The other media did not decrease below the original target pH of 4.0. There was a significant treatment effect observed in both runs of the experiment ($P < 0.001$ in both runs). In both runs, 100 and 500 ppm Fe amended media using iron sulfate heptahydrate suppressed *M. nivale*, however these treatments also led to a lower media pH over the course of the experiment, resulting in the previously mentioned confounding factor. Setting aside these treatments because of the observed media pH change, in run one, *M. nivale* was suppressed compared to the non-treated control by 500 ppm Fe amended using DTPA ferrous chelate. In run two, *M. nivale* was suppressed by 10 ppm amended using $FeSO_4 \bullet 7H_2O$, 100 ppm and 500 ppm Fe amended using DTPA ferrous chelate. In neither run of the experiment did $MgSO_4 \bullet 7H_2O$ suppress *M. nivale*, indeed often these amendments resulted in an increase in area of *M. nivale* compared to the non-treated control.

The effects of pH amended media using iron sulfate heptahydrate and lactic acid on the expansion of *Microdochium nivale in vitro*

A significant ($P < 0.001$) treatment effect was observed for both runs of this experiment. Similar to the previous experiment, the pH of the witness plates amended with iron sulfate heptahydrate decreased over the course of the 10 days of both runs of this experiment although the experimental units that included lactic acid were specifically chosen with this in mind (Table 5.7). The area of *M. nivale* on the media decreased as the lactic acid percentage or as the iron sulfate heptahydrate concentration increased, providing evidence that as pH decreases, the expansion of *M. nivale* also decreases. Comparing treatments with similar ending media pH, there is evidence that iron sulfate heptahydrate suppresses *M. nivale* for reasons other than a decrease in pH. Specifically, in run one, the 9898 ppm 1 M lactic acid amended media had an average pH of 3.4 at the beginning and also at the end of the experiment and these treatments had significantly more *M. nivale* growth than the 400 and 500 ppm Fe as $FeSO_4 \bullet 7H_2O$ amended media with an average start pH of 4.0 and an ending pH of 3.5 and 3.4 respectively. In run two, the 4975 ppm 1 M lactic acid amended media had an average starting pH of 3.7 and final pH of 3.6 with significantly more growth than the media amended with 200 or 300 ppm Fe as $FeSO_4 \bullet 7H_2O$ with an average start pH of 4.0 and 4.1 respectively and an ending pH of 3.6.

Discussion

This current field experiment did not find a benefit regarding Microdochium patch suppression by adding iron sulfate heptahydrate to phosphorous acid treatments. Phosphorous acid applied at 3.7 kg ha^{-1} every two weeks has previously been shown to suppress Microdochium patch on annual bluegrass putting greens (Dempsey et al., 2012) and there was even greater disease suppression observed when sulfur was applied at 12.2 kg ha^{-1} in

combination with 3.7 kg phosphorous acid ha^{-1} (Mattox et al., 2020). The modes of action of Microdochium patch suppression by sulfur or iron sulfate heptahydrate are yet to be explained, therefore it is difficult to speculate why sulfur and phosphorous acid combinations improve disease suppression and the same does not hold true for iron sulfate heptahydrate and phosphorous acid combinations. It is unlikely that the lack of benefit is tied to the rate of iron sulfate heptahydrate rates tested, because 106 kg $FeSO_4 \bullet 7H_2O$ ha^{-1} would provide the equivalent rate of 12.2 kg sulfur ha^{-1} and the 98 kg $FeSO_4 \bullet 7H_2O$ ha^{-1} tested in this experiment already completely suppressed Microdochium patch. The results of this experiment did support previous findings that when iron sulfate heptahydrate was applied alone, higher rates of iron sulfate heptahydrate suppress Microdochium patch more than lower rates (Mattox et al., 2016), although there was no significant disease suppression benefit observed when iron sulfate heptahydrate was added to phosphorous acid treatments.

Even though no benefit in Microdochium patch suppression was observed when iron sulfate heptahydrate was applied in combination with phosphorous acid in the field study, there was a benefit observed regarding turfgrass quality. Notably, rates of 24 or 49 kg $FeSO_4 \bullet 7H_20$ ha^{-1} applied in combination with phosphorous acid were often in the group with the highest turfgrass quality rating, even though these combinations did not result in acceptable turfgrass quality ratings in most months of the study. The decrease in turfgrass quality ratings was a result of the presence of Microdochium patch, turfgrass thinning, or unacceptable color because of a darkening of the turfgrass leaves following iron sulfate heptahydrate applications. Even though Microdochium patch severity was reduced when 12 or 24 kg $FeSO_4 \bullet 7H_20$ ha^{-1} was applied in combination with phosphorous acid compared to when iron sulfate heptahydrate was applied alone, the presence of a minor amount of disease or a blackening of the turfgrass leaves is

currently considered unacceptable for the majority of golf courses (Walsh, 2005). It is hypothesized that blackening of the turfgrass leaves may be caused by iron oxidation that deposits iron coatings on leaf surfaces. It is speculated that this color enhancement increases where iron reacts with leaf contents causing a dark pigmentation on leaf tips and other wounded areas of turfgrass leaves (Lennert, 1990; Watson, 2008). Evidence for iron oxidation is suggested from anecdotal reports that the blackening can be removed by soaking affected leaves in a reducing agent (Lennert, 1990). Increasing the water carrier volume of iron sulfate heptahydrate applications has been shown to increase green cover percentage compared to lower carrier volumes (Mattox et al., 2019). Perhaps repeating this field experiment using higher water carrier volumes may result in acceptable turfgrass quality when using combinations of iron sulfate heptahydrate and phosphorous acid.

Iron sulfate heptahydrate and phosphorous acid were shown to decrease the spray suspension pH compared to the well water used for mixing the suspensions. This observation lead to the desire to quantify the change in leaf surface pH over time resulting from the iron sulfate heptahydrate and phosphorous acid applications. Previous studies have explored measuring the foliar pH on multiple plant species including some graminoids such as wheat (*Triticum aestivum*), although the pH analysis required collecting leaves, oven drying them, mixing with deionized water and then testing the fluid (Cornelissen et al., 2006). In the interest of observing the change of foliar pH over time of grass plants growing in a growth chamber, the amount of material required to collect would have made the sample size cumbersome. For this reason, a flat-tip electrode pH meter was used to quantify the pH directly on the surface of the leaves. Flat tip electrodes have previously been used to test the pH of *Arabidopsis thaliana* leaf surfaces (Roelfsema et al., 1998) and in zoological studies to sample the pH of the surface of

marine tunicates (*Phallusia nigra*) (Hirose et al., 2001). This current study is the only known attempt to quantify the pH of the surface of a turfgrass sward. The advantage of this technique is that the pH of the turfgrass surface can be directly quantified over time. This is opposed to collecting foliar pH where the removal of leaves is required that could lead to potentially confounding the data with the leakage of intracellular components that possess a wide range of pH (Kurkdjian and Guern, 1989).

Microdochium patch suppression has previously been documented following the application of acidifying fertilizers such as sulfur (Brauen et al., 1975), although it was anecdotally reported in 1937 that the disease thrived when the conditions were acidic, although the soil pH was not specified (Jones, 1937). Spring applications of sulfur on colonial bentgrass (*Agrostis tenuis*) suppressed Microdochium patch although the date of disease assessment was not reported (Brauen et al., 1975). An increase in Microdochium patch has been observed up to 15 months after calcium carbonate was applied to annual bluegrass (Smith, 1959). These earlier studies may have led to current recommendations to maintain a lower soil pH to better manage Microdochium patch (Smiley et al., 2005). More recent studies have provided evidence that $FeSO_4 \bullet 7H_2O$ applied from 12 to 98 kg ha^{-1} (Mattox et al., 2016) or 12.2 kg S ha^{-1} applied every two weeks suppresses Microdochium patch (Mattox et al., 2020) and that monthly applications of either 12.2 or 24.4 kg S ha^{-1} reduced the number of fungicides necessary to mitigate Microdochium patch (McDonald et al., 2018). Because sulfur is applied so closely in time to the assessment of disease, it is unclear if the possible change in soil pH is affecting Microdochium patch or if the frequent applications of sulfur are directly affecting the fungus, the plant, or the environment (perhaps through a change in pH on the soil surface or among the turfgrass leaves as was hypothesized in these experiments).

The first growth chamber experiment found evidence that with few exceptions, the five rates of iron sulfate heptahydrate with or without phosphorous acid lowered the pH of the leaf surface compared to the well water control and that these effects lasted up to the last rating date (17 days post application). This observation supports the hypothesis that the decrease in the turfgrass surface pH may be creating an environment that is not as conducive for the growth of *Microdochium nivale* (Bennett, 1933) and that this is contributing to the Microdochium patch suppression observed by the same treatments in the field experiment. However, the second growth chamber experiment found that 98 kg $FeSO_4 \bullet 7H_2O$ ha^{-1} applied with or without 3.7 kg H_3PO_3 ha^{-1} consistently suppressed Microdochium patch while a range of treatments of H_2SO_4 did not suppress Microdochium patch. This study found that the pH of the leaf surface of the 0.5% v/v H_2SO_4 treated pots was always less than or equal to the pH of the $FeSO_4 \bullet 7H_2O$ treated pots while Microdochium patch was suppressed by $FeSO_4 \bullet 7H_2O$ and not by 0.5% v/v H_2SO_4. These results provide strong evidence that the suppression observed by iron sulfate heptahydrate was not solely (or perhaps not at all) caused by a decrease in pH of the leaf surface.

Exploring the growth chamber results through *in vitro* experiments provided evidence that chelated iron, and iron sulfate heptahydrate suppress *M. nivale in vitro*, although the effects of iron sulfate heptahydrate on media pH does leave open the possibility for pH to be a confounding factor and complicates the interpretation of the results. There is very little information in the literature about how iron sulfate heptahydrate affects media pH over time. There is evidence that iron hydroxides are formed in solution when iron sulfate is oxidized and the subsequent hydrolysis reactions leads to the production of hydrogen ions, resulting in a lower pH (Lin et al., 1996). In the soil matrix, it is known that iron sulfate reacts with water to produce hydrogen ions also leading to a reduction in pH (Carrow et al., 2001). It is probable that these

processes are also occurring in the potato dextrose agar media, although there is only a limited amount of references regarding the change in media pH over time. It is known that growth media pH is affected by heat sterilization (Skirvin et al., 1986), likely caused by the media chemistry being altered during exposure to the extreme temperatures (Behagel, 1971) and some researchers have compensated for this by testing the media pH while the media is still warm or by pushing the pH meter into the hardened media (Skirvin et al., 1986). Others have broken up the media, homogenized, and added sterile water to test the media pH (Wetzstein et al., 1994). In this current experiment, a flat-tipped electrode was used to test the pH of the media at room temperature because this was the temperature at which *M. nivale* was to be exposed (Harrigan, W., 1998). The inclusion of "witness" plates not exposed to the fungus allowed for periodic measurements of the growth media pH to occur throughout the study to quantify any changes over time. Similar to the study above (Lin et al., 1996), the pH of the iron sulfate heptahydrate amended media decreased over the course of the experiment. This was not observed with the other amendments in this study, which included chelated iron, lactic acid, magnesium sulfate heptahydrate, and ammonium hydroxide. Without the continual pH quantification, this potential confounding factor would have been missed, stressing the importance for similar studies and similar measurements over time to be considered in the future.

Notwithstanding the change in pH observed over time, these current experiments did provide evidence that both iron sulfate heptahydrate and chelated iron suppress the growth of *M. nivale in vitro*. It is not yet known how iron is affecting the growth of *M. nivale*, however iron-induced toxicity is a possible cause, with the Fenton reaction forming free radicals or competition of iron with other metals disrupting enzymatic reactions (Fenton, H., 1894; Vance and Miller, 1998; Gerwien et al., 2018). The effects by iron sulfate heptahydrate in these *in vitro*

experiments were only fungistatic, however. Plugs of *M. nivale* mycelium removed from the 500 ppm Fe amended with iron sulfate heptahydrate resumed growth on fresh unamended quarter strength potato dextrose agar with 48 hours (anecdotal data not included).

Conclusion

The field experiment demonstrated that there was no benefit in combining iron sulfate heptahydrate with phosphorous acid regarding suppression of Microdochium patch on annual bluegrass putting greens, however there was a benefit observed regarding turfgrass quality. Two growth chamber experiments demonstrated that the field experiment treatments were reducing the pH of the leaf surface compared to the well water control and that there is evidence that the reduction in pH does not explain all of the Microdochium patch suppression observed. Two *in vitro* experiments further supported these results, providing strong evidence that pH reduction of the leaf surface is not an effective means to suppress Microdochium patch on annual bluegrass putting greens. In the course of this experiment, the use of a flat-tipped pH meter was demonstrated to provide a means of quantifying the pH of the turfgrass leaf surface and the surface of the potato dextrose agar media. The use of this technique may be valuable to other researchers looking to test the effects of pH on plant surfaces or on solid media over time.

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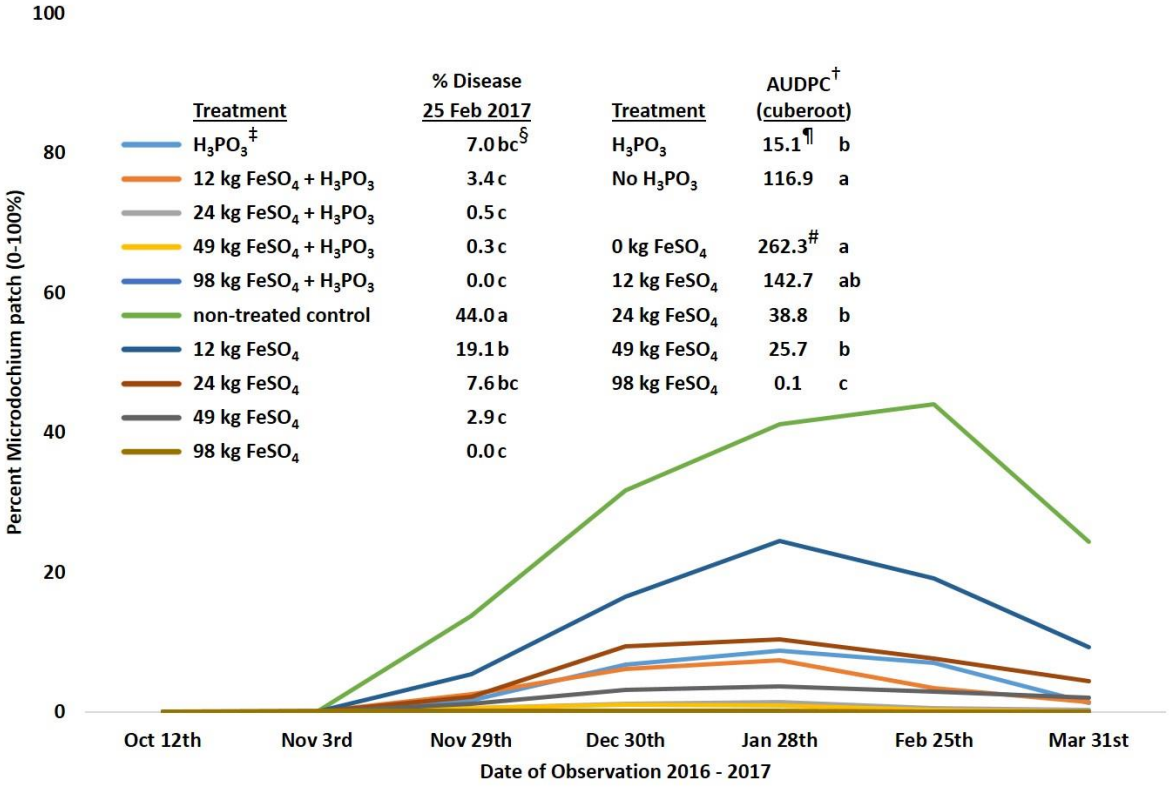

Figure 5.1. Effects of treatments on disease percentage and area under disease progress curve on an annual bluegrass putting green in Corvallis, OR. † Calculated from the beginning of the trial in year one (29 Sep. 2016) until the peak of disease (25 Feb 2017). ‡ H_3PO_3 = phosphorous acid applied at 3.7 kg H_3PO_3 ha^{-1}. § Means in the same column followed by the same letter are not statically significant according to Tukey's Honest Significant Difference at $P \leq 0.05$. ¶ Means are averaged across iron sulfate heptahydrate treatments and are back transformed from the cuberoot means. # Means are averaged across phosphorous acid treatments and are back transformed from the cuberoot means.

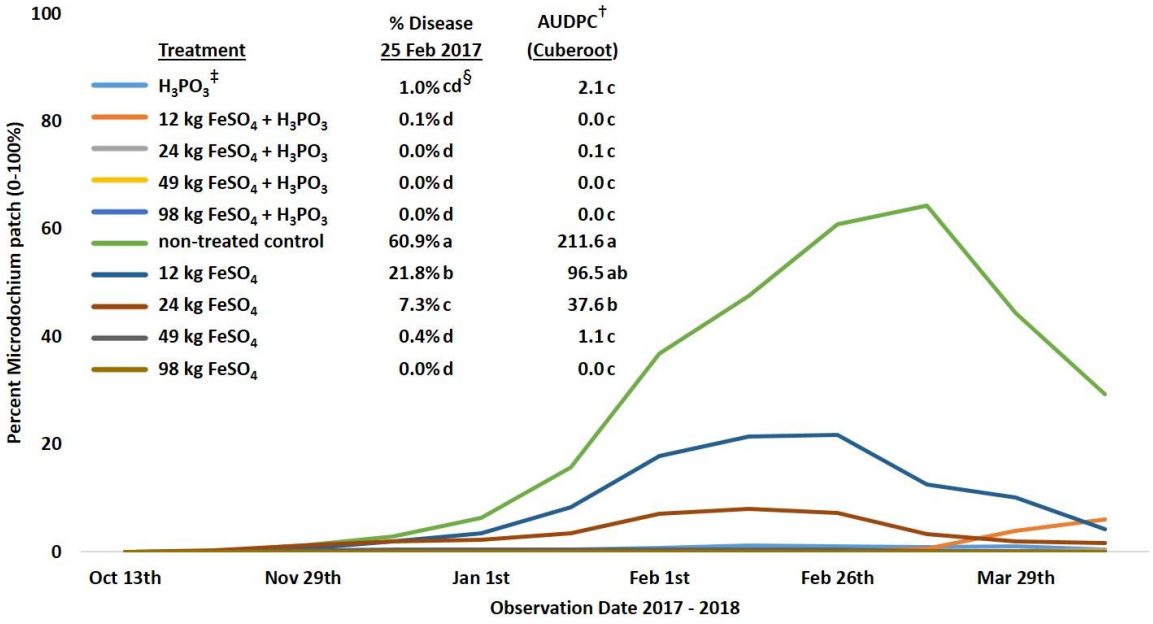

Figure 5.2. Effects of treatments on disease percentage and area under disease progress curve on an annual bluegrass putting green in Corvallis, OR. † Calculated from the beginning of the trial in year two (28 Sep. 2017) until the peak of disease (26 Feb 2018). ‡ H_3PO_3 = phosphorous acid applied at 3.7 kg H_3PO_3 ha^{-1}. § Means in the same column followed by the same letter are not statically significant according to Tukey's Honest Significant Difference at $P \leq 0.05$.

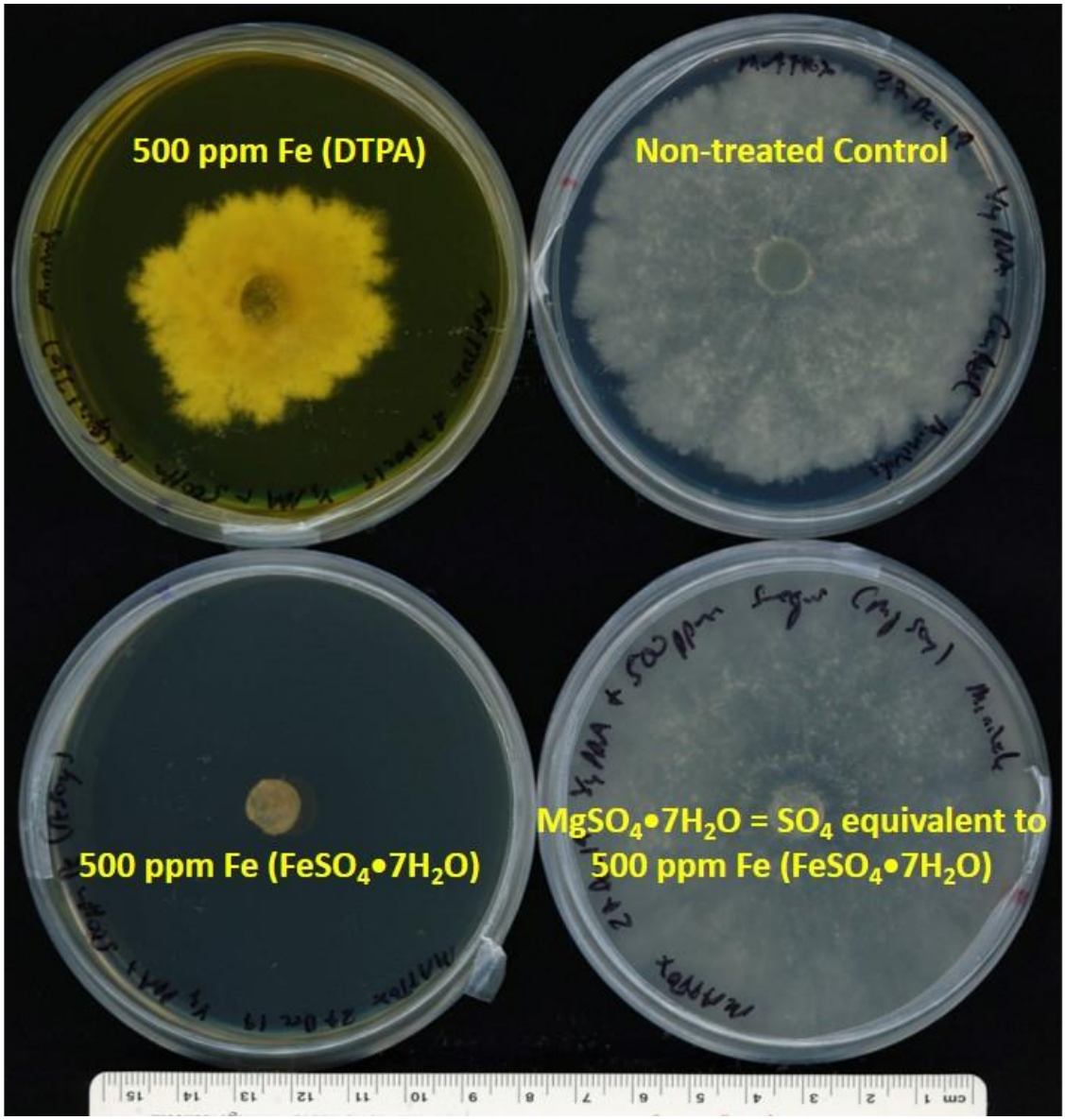

Figure 5.3. The effects clockwise from top right of the non-treated control, $MgSO_4$ applied at the SO_4 equivalent rate as found in 500 ppm Fe applied as $FeSO_4•7H_2O$, 500 ppm Fe applied as $FeSO_4•7H_2O$, and 500 ppm Fe applied as DTPA ferrous chelate.

		Year One - 2016 to 2017[†]					
		- Pr > F -					
	DF	29 Nov	30 Dec	28 Jan	25 Feb	31 Mar	27 Apr
Source of Variation	9	P=.005	P=.001	P<.001	P<.001	P<.001	P<.001
H_3PO_3[‡]		5.9 a[§]	5.0 ab	4.4 ab	3.5 abc	4.9 ab	5.0 ab
12 kg $FeSO_4$ + H_3PO_3		5.9 a	5.5 ab	4.8 ab	4.3 abc	4.8 ab	5.1 ab
24 kg $FeSO_4$ + H_3PO_3		6.0 a	5.8 a	5.3 a	5.3 ab	5.3 a	5.0 ab
49 kg $FeSO_4$ + H_3PO_3		5.9 a	5.5 ab	5.4 a	5.5 a	5.4 a	5.4 a
98 kg $FeSO_4$ + H_3PO_3		6.6 a	5.1 ab	2.0 b	2.5 bc	2.8 ab	3.6 ab
non-treated control		4.4 a	3.9 b	2.8 ab	2.0 c	2.8 ab	3.0 b
12 kg $FeSO_4$		4.9 a	4.0 ab	3.4 ab	3.0 abc	3.5 ab	4.5 ab
24 kg $FeSO_4$		5.4 a	4.9 ab	3.9 ab	3.9 abc	4.5 ab	5.1 ab
49 kg $FeSO_4$		5.4 a	5.0 ab	4.3 ab	4.5 abc	4.9 ab	5.1 ab
98 kg $FeSO_4$		6.8 a	4.1 ab	2.0 b	2.0 c	2.0 b	2.6 b

Table 5.1. Effects of treatments on turfgrass quality on an annual bluegrass putting green in Corvallis, OR. [†] Applications began on 29 Sep. 2016 and the trial concluded on 30 Apr. 2017. [‡] H_3PO_3 = phosphorous acid applied at 3.7 kg H_3PO_3 ha^{-1} [§] Means in the same column followed by the same letter are not statically significant according to Dunn's Test at $P \leq 0.05$.

		Year Two - 2017 to 2018[†]					
		- Pr > F -					
Source of Variation	DF	16 Nov	16 Dec	14 Jan	14 Feb	15 Mar	12 Apr
Treatment	9	P=.007[‡]	P<.001	P<.001	P<.001	P<.001	P<.001
H_3PO_3		6.6 a[§]	6.0 ab	5.6 ab	5.5 abc	5.3 ab	5.3 ab
12 kg $FeSO_4$ + H_3PO_3		7.5 a	7.4 a	6.6 a	6.1 abc	5.3 ab	3.9 ab
24 kg $FeSO_4$ + H_3PO_3		7.5 a	6.8 ab	6.0 a	6.5 a	6.1 a	5.4 ab
49 kg $FeSO_4$ + H_3PO_3		7.4 a	5.8 ab	5.3 ab	6.1 ab	6.0 a	5.8 a
98 kg $FeSO_4$ + H_3PO_3		7.0 a	5.0 b	2.3 b	3.0 abc	2.8 ab	3.4 ab
non-treated control		6.3 a	5.3 ab	4.0 ab	2.3 c	2.0 b	3.0 b
12 kg $FeSO_4$		6.8 a	5.5 ab	4.6 ab	3.3 abc	3.0 ab	3.5 ab
24 kg $FeSO_4$		6.6 a	5.4 ab	5.0 ab	3.8 abc	3.6 ab	3.9 ab
49 kg $FeSO_4$		7.0 a	5.6 ab	5.3 ab	5.5 abc	5.6 a	5.8 a
98 kg $FeSO_4$		7.0 a	5.0 b	2.0 b	2.5 bc	3.0 ab	3.5 ab

Table 5.2. Effects of treatments on turfgrass quality on an annual bluegrass putting green in Corvallis, OR. [†] Applications began on 28 Sep. 2016 and the trial concluded on 30 Apr. 2018. [‡] H_3PO_3 = phosphorous acid applied at 3.7 kg H_3PO_3 ha^{-1} [§] Means in the same column followed by the same letter are not statistically significant according to Dunn's Test at $P \leq 0.05$.

		< - Run One - Days post application - >																			
pH[†]		0		1		2		4		6		8		10		12		14		17	
2.3	3.8 kg H_3PO_3 ha^{-1}	3.7	de[‡]	4.0	bc	4.2	b	4.1	bc	4.0	bc	4.4	b	4.0	b	4.6	ab	4.6	ab	4.4	bc
2.2	12 kg $FeSO_4$ ha^{-1} + 3.8 kg H_3PO_3 ha^{-1}	3.1	f	3.5	d	3.4	e	3.7	bc	3.7	c	3.9	bc	3.7	b	3.8	c	4.0	ab	4.2	b
2.2	24 kg $FeSO_4$ ha^{-1} + 3.8 kg H_3PO_3 ha^{-1}	3.5	ef	3.8	cd	3.4	de	3.7	bc	3.8	bc	4.0	bc	3.8	b	3.9	bc	4.5	ab	4.0	b
2.2	49 kg $FeSO_4$ ha^{-1} + 3.8 kg H_3PO_3 ha^{-1}	3.5	ef	3.6	cd	3.5	de	3.5	c	3.5	c	3.7	c	3.5	b	3.9	bc	4.2	ab	4.1	b
2.2	98 kg $FeSO_4$ ha^{-1} + 3.8 kg H_3PO_3 ha^{-1}	3.5	ef	3.6	cd	3.6	cde	3.5	c	3.5	c	3.6	c	3.5	b	3.8	c	3.7	b	3.7	d
6.5	Well water control	5.7	a	5.5	a	5.4	a	5.3	a	5.3	a	5.3	a	5.3	a	5.2	a	4.9	a	5.3	a
3.5	12 kg $FeSO_4$ ha^{-1}	4.4	b	4.4	b	4.1	b	4.2	b	4.4	b	4.3	b	4.4	b	4.2	bc	4.6	a	4.4	b
3.1	24 kg $FeSO_4$ ha^{-1}	4.3	bc	4.1	bc	4.2	b	4.1	bc	4.0	bc	3.9	bc	4.0	b	4.1	bc	4.4	ab	3.9	b
3.0	49 kg $FeSO_4$ ha^{-1}	4.1	bcd	4.0	bc	3.9	bc	3.7	bc	3.8	bc	3.7	c	3.8	b	4.1	bc	4.1	ab	4.0	b
2.9	98 kg $FeSO_4$ ha^{-1}	3.9	cde	3.9	cd	3.9	bcd	3.6	bc	3.4	c	3.5	c	3.4	b	3.7	c	4.0	ab	3.7	cd

Table 5.3. Effects of iron sulfate heptahydrate and phosphorous acid treatments on annual bluegrass surface pH Run 1. [†] pH of spray suspension listed for reference only. [‡] Means in the same column followed by the same letter are not statically significant according to standard t-tests at $P \leq 0.05$.

		< - Run Two - Days post application - >																					
pH†		0		1		2		4		6		8		10		12		14		17			
2.3	3.8 kg H_3PO_3 ha^{-1}	4.8	b‡	4.5	b	4.7	b	4.6	b	4.9	ab	5.1	a	4.5	b	4.8	b	4.7	b	4.8	b		
2.3	12 kg $FeSO_4$ ha^{-1} + 3.8 kg H_3PO_3 ha^{-1}	3.8	c	3.5	d	3.7	d	4.3	bc	4.2	c	4.0	b	4.1	b	4.2	bcd	4.0	bc	4.2	b		
2.2	24 kg $FeSO_4$ ha^{-1} + 3.8 kg H_3PO_3 ha^{-1}	3.9	c	4.0	bcd	4.0	cd	4.3	bc	4.3	bc	4.4	b	4.1	b	4.5	bc	4.0	bc	4.5	b		
2.2	49 kg $FeSO_4$ ha^{-1} + 3.8 kg H_3PO_3 ha^{-1}	3.9	c	3.8	cd	3.8	cd	4.0	bc	4.2	c	4.1	b	4.1	b	3.8	d	4.0	bc	4.3	b		
2.2	98 kg $FeSO_4$ ha^{-1} + 3.8 kg H_3PO_3 ha^{-1}	3.8	c	3.8	cd	3.9	cd	3.9	c	3.9	c	4.0	b	4.0	b	3.8	d	3.8	c	4.1	b		
6.7	Well water control	5.9	a	6.0	a	5.8	a	5.7	a	5.6	a	5.5	a	5.7	a	5.7	a	5.6	a	5.6	a		
3.5	12 kg $FeSO_4$ ha^{-1}	4.8	b	4.4	bc	4.3	bc	4.4	bc	4.3	bc	4.4	b	4.6	b	4.6	b	4.5	bc	4.7	b		
3.1	24 kg $FeSO_4$ ha^{-1}	4.3	bc	4.2	bc	4.2	bc	4.3	bc	4.1	c	4.2	b	4.5	b	4.4	bc	4.5	b	4.8	b		
2.9	49 kg $FeSO_4$ ha^{-1}	4.2	bc	4.1	bc	4.0	cd	4.2	bc	4.2	bc	4.3	b	4.3	b	4.2	bcd	4.4	bc	4.5	b		
2.8	98 kg $FeSO_4$ ha^{-1}	3.9	c	3.9	cd	3.8	cd	3.9	c	3.8	c	4.0	b	4.2	b	4.0	cd	4.2	bc	4.3	b		

Table 5.4. Effects of iron sulfate heptahydrate and phosphorous acid on annual bluegrass surface pH Run 2. † pH of spray suspension listed for reference only. ‡ Means in the same column followed by the same letter are not statically significant according to standard t-tests at $P \leq 0.05$.

Run 1	pH spray suspension	pH + 6 hours		pH + 7 days		pH + 14 days		pH + 21 days		*M. nivale* (mm^2) + 21 days	
0.05% H_2SO_4	1.98[†]	4.2	b[‡]	4.5	a[‡]	4.5	ab[‡]	4.5	abc[‡]	635.2[§]	ab[¶]
0.125% H_2SO_4	1.7	3.6	cd	3.9	ab	4.0	ab	5.0	abc	976.0	a
0.5% H_2SO_4	1.4	2.9	e	3.7	b	3.8	b	4.3	c	501.1	ab
Water Control	7.0	4.9	a	4.7	a	5.1	a	5.2	abc	492.8	ab
98 kg $FeSO_4$ ha^{-1}	2.6	3.5	cd	3.7	b	4.4	ab	5.6	ab	0.6	cd
3.8 kg H_3PO_3 ha^{-1}	2.1	3.7	c	4.3	ab	4.5	ab	4.4	bc	106.4	bc
98 kg $FeSO_4$ ha^{-1} + 3.8 kg H_3PO_3 ha^{-1}	1.9	3.3	d	3.6	b	4.4	ab	5.6	a	0.0	d

Run 2	pH spray suspension	pH + 6 hours		pH + 7 days		pH + 14 days		pH + 21 days		*M. nivale* (mm^2) + 21 days	
0.05% H_2SO_4	2.0	4.1	bc[‡]	4.7	a[‡]	3.9	ab[‡]	4.2	a[‡]	1787.3	ab[#]
0.125% H_2SO_4	1.6	3.7	cd	4.2	ab	4.0	ab	4.6	a	1304.8	ab
0.5% H_2SO_4	1.4	3.1	e	3.7	bc	3.5	b	4.6	a	1789.2	ab
Water Control	7.0	4.9	a	4.6	a	4.9	a	4.5	a	2025.5	a
98 kg $FeSO_4$ ha^{-1}	2.7	3.6	d	3.7	bc	4.3	ab	5.0	a	0.0	b
3.8 kg H_3PO_3 ha^{-1}	2.2	4.2	b	4.5	a	4.4	ab	3.8	a	309.0	ab
98 kg $FeSO_4$ ha^{-1} + 3.8 kg H_3PO_3 ha^{-1}	1.8	3.4	de	3.5	c	4.7	a	5.0	a	0.0	b

Table 5.5. Effects of treatments on turfgrass surface pH and on Microdochium patch suppression. [†] pH of spray suspension listed for reference only. [‡] Means in the same column followed by the same letter are not statically significant according to standard t-tests at $P \leq 0.05$. [§] Means are back transformed from the cuberoot means. [¶] Means in the same column followed by the same letter are not statically significant according to Tukey's Test at $P \leq 0.05$. [#] Means followed by the same letter are not statically significant according to Dunn's Test at $P \leq 0.05$.

	Run 1				Run 2			
Treatment	media pH Run 1 Start	*M. nivale* (mm^2)		media pH + 14 days	media pH Run 2 Start	*M. nivale* (mm^2)		media pH + 14 days
10 ppm Fe ($FeSO_4$)	3.99	4363	bc[†]	4.03	4.02	3446	d	4.12
10 ppm Fe (DTPA[‡])	3.93	5205	ab	4.06	4.04	3933	c	4.17
SO_4 equivalent to 10 ppm Fe ($FeSO_4$)	3.88	4671	b	3.99	4.02	4285	c	4.01
100 ppm Fe ($FeSO_4$)	3.94	603	d	3.59	4.01	567	f	3.57
100 ppm Fe (DTPA)	3.94	3608	c	4.02	4.05	3191	d	3.98
SO_4 equivalent to 100 ppm Fe ($FeSO_4$)	3.91	5323	ab	3.99	4.05	4865	b	4.01
500 ppm Fe ($FeSO_4$)	3.92	0	d	3.44	3.97	0	g	3.47
500 ppm Fe (DTPA)	3.96	999	d	3.99	4.04	1312	e	4.01
SO_4 equivalent to 500 ppm Fe ($FeSO_4$)	3.92	5938	a	4.02	4.00	5599	a	3.99
non-treated control	3.94	4592	bc	3.95	4.05	3964	c	4.02

Table 5.6. The effects of iron sulfate heptahydrate, chelated iron, and sulfate equivalent applied as magnesium sulfate heptahydrate on *Microdochium nivale in vitro*. [†] Means in the same column followed by the same letter are not statically significant according to Tukey's Test at $P \leq 0.05$. [‡] DTPA = ferrous chelate.

	Run 1			Run 2		
Treatment	media pH Run 1 Start	*M. nivale* (mm^2)	media pH + 10 days	media pH Run 2 Start	*M. nivale* (mm^2)	media pH + 10 days
Control	5.3	5662 ab[†]	5.2	5.2	5553 a	5.2
500 ppm LA[‡]	4.7	5678 a	4.7	4.6	5542 a	4.6
999 ppm LA	4.4	4554 c	4.3	4.4	4464 b	4.4
4975 ppm LA	3.7	601 d	3.7	3.7	698 c	3.6
9898 ppm LA	3.4	166 e	3.4	3.4	139 d	3.4
14778 ppm LA	3.3	18 f	3.2	3.4	22 d	3.2
19608 ppm LA	3.3	0 f	3.1	3.2	0 d	3.2
10 ppm Fe[§]	5.0	5560 b	4.7	4.9	5476 a	4.7
100 ppm Fe	4.3	519 d	3.8	4.3	514 c	3.8
200 ppm Fe	4.2	158 e	3.6	4.1	138 d	3.6
300 ppm Fe	4.1	86 ef	3.5	4.0	66 d	3.6
400 ppm Fe	4.0	43 f	3.5	4.0	39 d	3.5
500 ppm Fe	4.0	13 f	3.4	3.9	7 d	3.5

Table 5.7. The effects of pH amended media using iron sulfate heptahydrate and lactic acid on the expansion of *Microdochium nivale in vitro*. [†] Means in the same column followed by the same letter are not statically significant according to Tukey's Test at $P \leq 0.05$. [‡] LA = 1 Molar Lactic acid [§] Fe amended as $FeSO_4 \cdot 7H_2O$

Conclusion

These research experiments demonstrate that different combinations and frequencies of iron sulfate heptahydrate, phosphorous acid, sulfur, and mineral oil suppress Microdochium patch on annual bluegrass putting greens. Prior to these experiments, all of these different products were previously shown to suppress Microdochium patch, however no product completely suppressed disease or when disease suppression was observed, this was accompanied by turfgrass thinning. These experiments have primarily focused on different field studies to compare combinations, rates, and application frequencies of these products in order to suppress Microdochium patch and to maintain acceptable turfgrass quality. The motivation for this research was to inspect multiple avenues to suppress Microdochium patch using products that may be permitted where pesticide restrictions or fungicide resistance concerns exist.

Specifically, these research experiments provided evidence that applying mineral oil in combination with sulfur either every two weeks or every four weeks resulted in a loss of green cover percentage indicating that these combinations should not be applied under similar conditions. Research focusing on mineral oil and phosphorous acid combinations found that lower than previously tested rates and application timings suppressed Microdochium patch, even though complete suppression of disease was not observed. The reduction in frequency of mineral oil applications in combination with phosphorous acid in the winter months suppressed Microdochium patch although turfgrass quality was considered unacceptable in at least one month during the winter period. Studies focusing on iron sulfate heptahydrate applications found that applications applied at frequencies greater than every two weeks did not suppress disease as well as the two-week application frequency. Testing water carrier volumes ranging from 1017 to 4075 L ha^{-1} of 98 kg $FeSO_4 \cdot 7H_2O$ ha^{-1} applied every two weeks found that there were no differences observed regarding the suppression of Microdochium patch although higher water

carrier volumes did result in greater green cover percentage. No benefit was observed in adding iron sulfate heptahydrate to phosphorous acid applications regarding suppression of Microdochium patch, although turfgrass quality was improved at some rates of iron sulfate heptahydrate. The level of Microdochium patch suppression and the resulting turfgrass quality observed in these multiple field studies provides novel information for turfgrass managers looking to find alternatives to traditional fungicides to mitigate Microdochium patch on annual bluegrass putting greens. In areas where pesticide restrictions occur, perhaps stakeholders in those areas will tolerate a decrease in turfgrass quality compared to the over 40% Microdochium patch that was frequently observed in the non-treated control plots in these field experiments.

In the course of this experiment, the use of a flat-tipped pH meter was demonstrated to provide a means of quantifying the pH of the turfgrass sward and the surface of the potato dextrose agar media. Using this technique, a growth chamber experiment found that both iron sulfate heptahydrate and phosphorous acid applications reduced the turfgrass surface pH for up to 17 days post application. A subsequent growth chamber and two *in vitro* studies provided evidence that this reduction in turfgrass surface pH was not responsible for the suppression of Microdochium patch observed by iron sulfate heptahydrate in the field experiments.

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