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Title: Managing Microdochium Patch Using Non-Traditional Fungicides on Annual Bluegrass Putting Greens

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Currently, fungicide applications are the predominant method of control for the cool weather pathogen Microdochium patch (*Microdochium nivale*). Increasing pesticide restrictions have generated concern regarding management of Microdochium patch. Three separate field trials exploring non-traditional fungicides were conducted between 2013 and 2015 on an annual bluegrass (*Poa annua* L.) sand-based putting green at the Lewis Brown Horticulture Farm, Corvallis, OR. The objective of the first project was to evaluate the effects of the cultural practice of rolling in combination with mineral oil and fertility on Microdochium patch incidence. The objective of the second trial was to quantify the effects on Microdochium patch incidence using biological control products in combination with rolling. Finally, the objective of the third experiment was to quantify the effects of different nitrogen and iron sulfate rates in combination with simulated golfer traffic on the effects of Microdochium patch incidence as well as turfgrass recuperation. The first experiment determined that rolling in combination with Civitas One or Sulfur DF + PK Plus suppressed disease to levels comparable to traditional fungicides. Civitas One with rolling resulted in abiotic damage. The

second experiment determined that rolling as well as the biological control agents BW136N, followed by Rhapsody suppressed Microdochium patch disease. The third experiment determined that 4.88 Kg N ha^{-1} combined with 97.65 Kg $FeSO_4$ ha^{-1} provided the greatest combination of disease control and turf quality.

Managing Microdochium Patch Using Non-Traditional Fungicides on Annual Bluegrass Putting Greens

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

Clint M. Mattox, Author

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Introduction

Golf course putting greens are often compared to billiard tables, because the golf ball rolls along the surface similar to a billiard ball does on a slate table. Much like in billiards, dents or dings in the surface are not considered acceptable to golfers seeking to putt their golf ball from one section of the green to the golf hole. Turfgrass diseases are a concern for turfgrass managers as they have the capacity to damage the putting green, creating imperfections that are distracting visually and can also lead to conditions that cause a physical change in the consistency of the putting surface.

One disease that is particularly problematic on annual bluegrass, the prominent putting green turf in the Pacific Northwest, is Microdochium patch. Microdochium patch is a turfgrass disease that occurs in the cool humid winter months often when turfgrass is not growing to its full potential and it can be very damaging. Frequently, injury caused by Microdochium patch will persist for several months until temperatures rise to levels more conducive for vigorous turfgrass growth. To date, Microdochium patch has been mitigated with the use of fungicides that either inhibit or kill the fungus responsible for disease. However, pesticide use, including fungicides, has come under scrutiny in recent years and as a result, there is a desire to find alternative means of managing plants through the use of products that are considered less harmful for humans and the environment. To date, limited research has focused on managing Microdochium patch through alternative channels. Due to the industry demand to research alternative control techniques, three field studies were implemented in Corvallis, OR between 2013 and 2015 concentrating on three different areas of alternative disease control.

The first study focused on the use of three different alternative products that had previously shown some potential for Microdochium patch reduction and the cultural practice of rolling. The second study looked at the effects of three different biological control products in combination with rolling on the incidence of Microdochium patch. A third field study looked at how different rates of nitrogen and iron sulfate in combination with simulated golfer traffic affected Microdochium patch disease development and turfgrass recuperation during the winter months.

Chapter 1

Literature Review

Introduction

Microdochium patch is a turfgrass disease caused by the fungal pathogen *Microdochium nivale* (Fr.) Samuels & Hallett, pronounced [micro-doke-ium] [nee-VAH-lee] (Vincelli and Munshaw, 2014). Microdochium patch is one of the most prominent turfgrass pathogens in the Pacific Northwest and Northern Europe (Vargas, 2005). While this pathogen can infect all cool-season grasses it is particularly damaging to annual bluegrass (*Poa annua* L.), the prominent putting green turf in the Pacific Northwest. In order to provide golf course putting surfaces that live up to the expectations of golfers, superintendents have successfully used fungicides in order to manage this otherwise devastating disease (Vincelli and Munshaw 2014). However, with new environmental legislation and public awareness campaigns, superintendents are inclined to look into alternative control measures (Christie, 2010).

Indeed, in the past few years there has been new legislation regarding the reduction of pesticides for turfgrass management. A current example is the cosmetic ban on lawn care in Canada that restricts the use of pesticides on lawns for aesthetic purposes (Health Canada, 2011). After the implementation of this ban, the Minister of the Environment in Canada, John Wilkinson, stated; “The rapid decline in levels of pesticides now banned for lawn care use is a strong example of this ban’s success. This is great news for Ontario families and for the environment” (Wilkinson, 2011).

In order to prepare for possible future restrictions concerning turfgrass management using chemicals, much research has focused on finding alternatives to chemical use for turfgrass pests. To date however, nothing in the lines of alternative controls has been found to be adequate at eradicating or preventing the diseases caused by the turfgrass pathogen, *M. nivale*. The fact that suitable alternatives do not yet exist, has led turfgrass managers to continue to use chemical fungicides to control this pathogen.

A Brief Overview of *Microdochium nivale*

M. nivale is the pathogen responsible for the turfgrass diseases Microdochium patch and pink snow mold. The disease is referred to as pink snow mold when it occurs in relation with snowfall and as Microdochium patch when it occurs in the absence of snow cover. The common name of pink snow mold derives from the mycelium that ironically is white, however upon exposure to light the mycelium and sporodochium (mass of hyphae that bears the conidiophores where the asexual conidia are formed) production is of a pinkish color, hence the name pink snow mold (Smiley et al., 1992; Hsiang, 2009). This literature review and subsequent chapters will focus on Microdochium patch.

Microdochium patch is a fungal disease that develops on many different types of turfgrasses; annual bluegrass, creeping, velvet, and colonial bentgrass (*Agrostis stolonifera, A. canina, and A. tenuis*, respectively), Kentucky bluegrass (*Poa pratensis*), perennial ryegrass (*Loium perenne*) as well as fine and tall fescue (*Festuca* spp., and *Festuca arundinacea*) (Vargas, 2005). Because of the cool, rainy, moderate environmental conditions of the Pacific Northwest and British Columbia, annual bluegrass is the predominant turf species on putting greens (McIver, 1997; Cook, 2008). These environmental conditions are also ideal for the development of Microdochium patch (Vargas, 2005). Indeed, Microdochium patch thrives in cool, moist weather with or without snow cover, which is commonplace in the Pacific Northwest for more than 6 months of the year. Therefore, the presence of a particularly susceptible host and the ideal environmental conditions for *M. nivale* development make this pathogen the major concern of the typical golf course manager in the Pacific Northwest.

Identification of the disease in the field is fairly straightforward. It is one of a handful of diseases that occur in cool, wet weather and the reddish-brown border on the edge of diseased patches can easily be distinguished from other diseases. This is especially true on annual bluegrass putting greens, where the reddish-brown border is particularly prevalent. The patches start off small; around 5 to 7.5 cm in diameter and can appear water-soaked in the field (Smiley et al., 1992). If no fungicide applications are made, these patches can expand to 30 cm in diameter in a few days. *M. nivale* does not usually kill the plant and often times the turf in the center of the patch of disease will begin recovering while the plants on the periphery continue to be affected, producing a frogeye appearance (Couch, 1995).

The pathogen *M. nivale* is most active in vitro at temperatures around 20 degrees Celsius as shown by Dwyer (2004) as well as Endo (1963). However, in the field the disease is rarely present at these temperatures, most likely due to the fact that the turfgrass is actively growing under these conditions and is able to defend itself against disease. Dwyer (2004) found that Microdochium patch outbreaks in the field were most common between 8 and 17°C and when there was more than 20 hours of 90% or greater relative humidity.

Microdochium nivale grows vegetatively through the production of hyphae. The dividing wall called a septate which separates the hyphae and the absence of clamp connections aids in its identification under a microscope. When the weather is cool, a small mass of hyphae called a sporodochia is formed from which the asexual spores, conidia, will develop. The conidia of *M. nivale* are crescent shaped and have one to three septations. (Smiley et al., 1992) *M. nivale* can act as a saprophyte (Vargas, 2005) and has been shown to survive up to one year in infected straw either buried or on the soil surface. *M. nivale* can be spread by mycelium which has infected debris in the turf canopy or by germinating conidia which are put into contact with susceptible leaves (Tronsmo et al., 2001).

Dahl (1931) found that the mycelium of *M. nivale* did not penetrate the epidermal cells of the leaves, but grew along the leaf surface and entered the leaves through the stomata. According to Couch (1995), *M. nivale* can also penetrate through cut leaf tips and through epidermal cells that have undergone injury, however it will not enter healthy epidermal cells. When Diamond and Cook (1997) studied *M. nivale* under a scanning electron microscope, they found that it took 24 to 48 hours for conidia to germinate and to observe hyphae running adjacent to the leaf's epidermal cell walls. Stomatal colonization did not occur immediately, it took 4 to 5 days after the date of infection.

Annual Bluegrass

Poa annua is a difficult plant to describe given the presence of multiple biotypes in a given area (Vargas and Turgeon, 2004; Cook, 2008). In addition, annual bluegrass expresses two different life cycles; annual and perennial. Both plants are more prone to *M. nivale* than bentgrass, the predominately seeded turfgrass during green construction in temperate climates (Christians, 2007).

The annual biotype is *Poa annua var. annua*. It is commonly referred to as annual bluegrass or meadowgrass. This biotype is a cool season grass that is adapted to nearly all regions of the planet, indeed it is found on all 7 continents (Huff, 2004). It is often considered a weed due to its winter annual life cycle, which results in poor drought tolerance and associated seed heads flushes. The true winter annual dies after the spring seed production (Beard, 1973).

There is another biotype that also produces seeds in the spring and generally whenever it is under stressful conditions, yet survives as a perennial when managed properly throughout the summer. This biotype is *Poa annua* var*. reptans* (Christians 2007). It is found on highly manicured closely mown turfs. It possesses denser tillers, has a finer leaf texture, a darker color, and lower seed head production than the annual biotype (La Mantia and Huff, 2011). Recent research suggests that the annual biotype can transition into a perennial biotype under high maintenance conditions (La Mantia and Huff, 2011). The perennial biotype is the dominant biotype on golf course putting greens in the Pacific Northwest.

Even though a great deal of effort has been dedicated to the eradication of all biotypes of annual bluegrass, it has proven to be a hearty fighter and most turf sites in the Pacific Northwest convert naturally from bentgrass to *P. annua* over time. Truly, most of the finest golf courses in this area consist of predominately *P. annua* turf. For this reason, we will focus on the management of *M. nivale* in regards to its presence on annual bluegrass.

Legislation Limiting Pesticide Use in Turfgrass Management

The need for alternative control options for turfgrass diseases is becoming apparent as a result of new legislations worldwide concerning the use of pesticides on amenity turf. In the United States, as of July 01, 2010, no pesticides can be applied on turf at day cares or K-8 schools in the state of Connecticut (Connecticut's Lawn Care Pesticide Ban, 2010). In San Francisco, there has been a restriction on pesticides allowed on city and public property since 1996, although at least three synthetic fungicides were still authorized as of 2015 on golf course putting greens (San Francisco Environment, 2015). Another example of pesticide restrictions are buffer zones around waters that support Pacific Salmon that have been in place since 2004 in Oregon, Washington and California (US EPA, 2015).

In Europe the pesticide laws in regards to turfgrass management differ greatly from country to country. For example, in France there are many chemistries available for turfgrass management (Ephy, 2015), but the laws regarding application are difficult. Nearly all synthetic products have a re-entry interval minimum of between 6 and 48 hours, which are very difficult to abide to in a golf course setting (Ministère de l'agriculture et de la pêche, 2006). In addition, there is a nationwide objective in France to reduce pesticide use by 50% by 2018 (Philpott, 2012).

In other European countries, there are less products available, only three fungicides are available in Norway and Sweden, four in Holland and three in Germany with the possibility of others under special situations when given permission by the local government (Personal communication with local turfgrass professionals; Bundesamt fur Verbraucherschutz und Lebensmittelsicherheit, 2015). In all of these countries, the chemistries available are very similar to each other leading to concerns about the potential for resistance development.

In Canada, a recent example of pesticide restrictions is the cosmetic pesticide ban in Ontario that took effect on Earth Day April 22, 2009 which forbids the use of pesticides for cosmetic purposes (Christie, 2010). For the moment, golf courses in Canada are permitted to use synthetic pesticides for cosmetic purposes, but it is likely stricter controls will come in the future (Pesticides Act, 2015).

Even though effective chemical control options for managing turfgrass diseases in the USA still do exist (Vincelli and Munshaw, 2014), there is a great deal of research going on regarding alternative control methods. Beyond the scope of changes in legislation, there are many different reasons that turfgrass managers are motivated to look into alternative controls as a way to manage disease. Primarily, with an increase in frequency of fungicide additions of the same chemistries, there is a greater chance of chemical resistance occurring (Latin, 2011). If alternative methods can provide a means to decrease chemical application frequency, perhaps fungicide resistance can be circumvented or delayed. Another major motivator for alternatives to chemical use is the public image of golf and how it is perceived in regards to being a benefactor to the environment (Nelson, 2005; Attorney General of NY, 1995). Finally, some turf managers have no choice but to seek alternative controls as laws and regulations have become more stringent and there may be no more chemical controls available (Barton, 2008).

A recent example is the Vineyard Golf Club located on the island of Martha's Vineyard, MA and managed by environmental award winning Superintendent Jeff Carlson. This golf club is 100% organic, largely due to the fact that Martha's Vineyard County Commission stipulated prior to construction that any golf course that was to be built on the proposed site could not use pesticides or synthetic fertilizers (Barton, 2008). By necessity, Mr. Carlson has been experimenting with many management techniques, including organic fertilization, biological control products, alternative products, dew removal, hand weeding, etc. (Carlson, 2010). The members and visitors that include diplomats and presidents hold him to a high standard. Indeed, President Obama is known to play here when on retreat (Pennington, 2010).

Potential Fungicide Alternative Control Methods

The following sections of the literature review will focus on four major avenues of alternative controls for the management of *M. nivale*; cultural practices, fertility products, biological control products and mineral oils.

Cultural Practices

Research shows that Microdochium patch development is most prevalent under moist conditions and the spreading of the fungus through leaf contact can be favorable under drizzling rain (Smiley et al., 1992). It has been suggested that mowing late into the season is important as long leaves tend to become matted down, which can result in areas of high humidity prone to *M. nivale* invasion (Smiley et al., 1992). Cultural practices to reduce Microdochium patch pressure include improving drainage, controlling thatch, improving air circulation and removing dew in order to accelerate drying (Ellram et al., 2007; MacDonald, 1999).

Another recent cultural practice being implemented in disease control is the action of rolling. Research conducted in the Midwest has shown that rolling significantly reduces dollar spot *(Sclerotinia homeocarpa*) (Giordano, 2013). Even more promising data suggests that the positive effects of rolling in disease suppression is cumulative year after year. Giordano (2013) observed significantly better turfgrass with less disease after two and three years of rolling in comparison to a single year of rolling. The benefits of rolling are being so well accepted by the industry that the company Salsco has introduced a fairway roller designed to give the same benefits to fairways as have been noted on golf greens (Williams, 2012).

Intriguingly, the study at MSU concluded that the disease suppression effects were not a result of the removal of dew, as rolling had equivalent effects if it was preformed in the morning or in the afternoon (Giordano et al., 2012). An alternative hypothesis proposed for this suppression in disease was that rolling was having an effect on the dynamics of the substrate microbial population. To test this hypothesis, phospholipid fatty acid analysis data was taken and the results showed that rolling resulted in an increase in the bacterial population when compared with the controls. The hypothesis is that the increase in bacterial populations is having an effect on the pathogen populations. The effect is unclear at the moment, however the researchers suggest that the bacteria could be directly competing for the same resources, antagonizing the fungal pathogen, acting as a parasite on the fungal pathogen or by causing induced resistance in the plant (Giordano, 2013).

Researching further into the effects of dew removal in regards to disease suppression, there is data that continues to support the long accepted theory that dew removal does indeed have a positive effect on the incidence of some fungal pathogens. Studies have shown that removal of dew has been able to increase the performance of fungicides and thus reduce the total number of applications necessary for control of dollar spot (Delvalle et al., 2011). The reasoning behind this accepted practice is that the removal of dew disrupts the leaf wetness duration necessary for infection, but also removes or displaces the developing fungal hyphae. In addition, it is accepted that the morning moisture on the leaf surface is a mixture of dew and guttation material consisting of amino acids and sugars that can aid the development of the fungal growth (Delvalle et al., 2011).

Fertility Products

Fertility recommendations regarding Microdochium patch have primarily concerned nitrogen and sulfur applications (Brauen et al., 1975). Regarding nitrogen fertilization, it is a common practice to apply light frequent amounts of nitrogen to golf course putting greens throughout the growing season. Rates tend to vary from extremely light amounts to around 25 grams of nitrogen per square meter per growing month. It the case of Microdochium patch it is recommended to avoid lush turf going into the winter, therefore golf course superintendents reduce nitrogen applications in mid-Autumn in order to prepare the turf for the colder months (Couch, 1995).

Another potential candidate for disease suppression with fertilizer is the use of sulfur or sulfur containing products such as iron or ammonium sulfate to decrease pressure from fungal pathogens. A recent study in the Pacific Northwest has shown that sulfur applications had a positive effect on Microdochium patch suppression (Golembiewski et al., 2009). It is hypothesized that acidifying the thatch and plant tissue may have an affect on the disease (Baldwin, 1989).

Phosphite products are often sold as fertilizers, yet they have also been shown to possess disease suppressive characteristics. There are many phosphite products being promoted commercially and university test results have been published regarding the efficacy of these products (Dempsey et al. 2012). Research at Oregon State University concluded that phosphite products provide *M. nivale* suppression when disease pressure is low and potentially reduce the number of annual fungicide applications necessary to control the pathogen (Golembiewski and McDonald, 2011a). It is speculated that the mode of action of the phosphite products depends on where and how it is associated with the host plant (McDonald et al., 2001). Research shows that when phosphite is concentrated in the root, it inhibits growth of the pathogen by directly acting upon it. However, when the concentration in the roots are low, it indirectly suppress the pathogen by stimulating defense enzymes in the host plant (Jackson et al., 2000).

Biological Control Products

Biological control can be defined as the complete or partial demise of plant pathogen populations through the use of other living organisms (Agrios, 2005). In regards to plants, the influence that the microbes have on disease can be summarized by three main processes; antiobiotic biosynthesis where specific microbes produce antibiotic compounds that suppress pathogens, resource competition where the microbes compete with the pathogen for the same resources and parasitism where microbes attack other microbes (Nelson, 2003). In turfgrass, biological control has mainly been used in three different ways; microbes thought to have a positive influence on disease suppression can be added to the concerned site, organic matter can be added to stimulate the growth of existing microbes leading to an expansion of their population and finally through the addition of composts that possess both microbes and organic matter.

Twelve years ago, Cornell plant pathologist Eric Nelson published a complete list of commercial turf disease biological control products available in the USA. There were six at the time, *Trichoderma harzianum* (commercially available as Turf Shield), *Gliocladium catenulatem* (Primastop), *Pseudomonas aureofaciens* (Spotless), *Streptomyces lydicus* (Actinovate), *Bacillus subtilis* (Companion), and *Bacillus licheniformis* (Ecoguard) (Nelson, 2003). More than a decade has passed and the amount of biological control products labeled for turf has actually decreased. Dr.

Nelson attributes the lack of products available to four reasons; fungicides are very effective leading to a lack of interest by the turfgrass professional, fungicides are more user friendly as they can be stored for long periods and are easier to apply, fungicides are active over a broad range of environmental conditions and finally companies are hesitant to make large investments in a niche industry that is uncertain at best (Nelson, 2003).

Past research has shown that there is a potential for biological controls regarding the management of Microdochium patch. Testing performed by Carmen Raikes in 1997 in the UK obtained results showing that *Enterobacter cloacae* suppressed *M. nivale* by more than 43% a week after inoculation with *M. nivale* (Raikes, 1997). Further studies showed disease suppression greater than 70% by three different bacteria tested; *Bacillus sp*. resulted in 76.1% suppression of *M. nivale* with *P. aeruginosa* and *P. cepacia* each resulting in 70.1% suppression*.* *Chaetomium globosum* was also tested showing promising results (Raikes, 1997).

Perhaps the most promising biological control to be released in recent years was *P. aureofaciens TX-1* released under the name Spotless. Research at Michigan State University showed a reduction in both *S. homeocarpa* and *M. nivale* incidence and a company called Eco Soils System Inc. licensed the product and marketed a field bioreactor called the bioject that could inject the bacteria through the irrigation system, applying them over the entire course during the evenings (Horvath and Vargas, 2000). Unfortunately, when implemented in the field, the results did not prove to be as optimistic as in the study (Hardebeck et al., 2004).

Research involving composts and disease suppression yield positive results, however not all composts are equal in regards to reducing disease (Horvath and Vargas, 2000). When composts do show suppressive qualities, Dr. Nelson attributes these virtues to the fungal and bacterial populations increasing in the soil or that the compost serves as a food source for the microorganisms that already exist in the soil (Nelson, 2003). This argument is supported by research that suggests composts are a much more suitable medium for microbial populations survival than the traditional sphagnum peat used in pots and golf course putting greens (Hoitink and Boehm, 1999).

In trials implemented by Boulter (2002), composts were able to suppress disease when pressure was low, in addition composts resulted in faster green-up the following spring when compared to fertilizers and fungicides. Boulter concluded that composts act to suppress turfgrass disease through both physiochemical and biological means. The physiochemical facets include characteristics involving nutrient availability, organic matter content, acidity of the soil, affects on moisture, and other influences. Nutrient competition with pathogens, lytic enzyme production, parasitism and host defense responses are some of the biological facets scientists speculate that composts can provide (Boulter et al., 2002).

Even though the disease suppression qualities of composts are often inconsistent in their levels of controls, Noble and Coventry (2005) point to research where disease control was achieved with compost additions, however the same compost lost its disease suppressive qualities following sterilization. This led to the conclusion that the disease suppressive qualities were more biological than physical or chemical in character.

Even though biological control products and various applications of compost have shown potential for disease suppression, they are still unable to compete with the efficiency of fungicides in regards to disease suppression and therefore remain a marginal choice for turf managers.

Mineral Oils

A mineral oil product called Civitas has had commercial success due to its reported suppression of *S. homeocarpa* and potential effects on *M. nivale* (Cortes-Barco et al. 2010a; Nash, 2011) This mineral oil is thought to have an influence on the plant defense activators and therefore better prepares the plant against fungal attack (Cortes-Barco et al. 2010b).

Civitas One is an Organic Materials Review Institute (OMRI) registered turfgrass fungicide that consists of a food-grade emulsifier and synthetic isoparaffin (Hsiang et al., 2013) in addition to the green pigment Harmonizer containing polychlorinated copper (Cu) II phthalocyanine (Nash, 2011). Civitas and Harmonizer have been shown to suppress *M. nivale* when tested in vitro (Nash, 2011) and greenhouse studies have shown that when applied to the soil, Civitas at 10 and 20% concentration suppressed Microdochium patch between 50 and 65% respectively on creeping bentgrass (Cortes-Barco et al., 2010a). Disease control through soil-based applications provide evidence that Civitas is influencing either the soil or soil microorganisms that leads to an induced systemic response in turfgrass (Cortes-Barco et al., 2010a and 2010b). Nash (2011) concluded similar results regarding experiments using a 5% solution of Harmonizer applied to the soil that resulted in a 32% suppression of dollar spot (*S. homeocarpa*) on creeping bentgrass.

Other field studies have shown that Civitas + Harmonizer provide control of anthracnose (*Colletotrichum cereale*) (Aynardi, 2011b; Popko and Jung, 2012) on a mixed creeping bentgrass and annual bluegrass putting green. Civitas alone provided control of drechslera leaf spot/melting out (*Drechslera poae*) on Kentucky bluegrass (*P. pratensis*) (Uddin et al., 2010) and dollar spot on a mixed stand of creeping bentgrass / annual bluegrass (Aynardi et al., 2012b) and Gray leaf spot (*Pyricularia grisea*) on perennial ryegrass in Pennsylvania (*Lolium perenne*) (Aynardi et al., 2012a). However, Civitas in combination with Harmonizer did not control gray leaf spot (*Pyricularia grisea*) on perennial ryegrass in Virginia (McCall and Focht, 2010), dollar spot on creeping bentgrass in North Carolina (Soika et al., 2011) or in Oklahoma (Smith and Walker, 2012) or Drechslera leaf spot / melting out on Kentucky bluegrass (Aynardi et al., 2011a). While multiple studies have been performed on a variety of turfgrass diseases and species, work concerning Microdochium patch on annual bluegrass is limited.

Conclusion

In conclusion, all the indications are showing that the future of golf course management is moving towards a reduction in pesticide use. In regards to *M. nivale* on *P. annua* putting greens, there are a lot of options that currently exist that can reduce fungal pathogen pressure, however none have been shown to be as reliable as traditional synthetic fungicide controls. Further research is warranted in order to find alternative means of Microdochium patch control on *P. annua* putting greens that compares to the effectiveness of synthetic fungicides. If stricter regulations are put into place and no effective alternative practices are discovered, the turfgrass manager and the golfer will be compelled to accept lower expectations and less control in regards to putting green quality.

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Chapter 2

Controlling Microdochium Patch in the absence of Traditional Fungicides using Mineral Oil, Fertility Products and Rolling

Abstract:

Microdochium patch is a turfgrass disease in cool, humid regions caused by the pathogen *Microdochium nivale* (Fr.) Samuels & Hallett. Currently, fungicide applications are the predominant method of control. Increasing pesticide restrictions have generated concern regarding management of Microdochium patch. The objective of this research was to evaluate the effects of the cultural practice of rolling in combination with mineral oil and fertility treatments in the absence of traditional fungicides. A two-year field trial was initiated in September 2013 and concluded in June 2015 on a sand-based putting green at the Oregon State University Lewis Brown Horticulture Farm, Corvallis, OR. Experimental design was a 2 by 2 by 4 by 2 randomized complete split-plot design with four replications. Factors included year, rolling, fertility and mineral oil treatments. Rolling treatments were applied five days a week and fertility and mineral oil treatments were applied every two weeks from September 26, 2013 to June 13, 2014 and again from September 22, 2014 to June 12, 2015. No traditional fungicide treatments were applied during the study. The three fertility treatments were; Sulfur DF 12.22 Kg S ha^{-1}, PK Plus 3.66 Kg H_3PO_3 ha^{-1} and Sulfur DF+PK Plus. Mineral oil treatments were Civitas One 19.94 Kg a.i. ha^{-1} or no Civitas One. Turf quality (1-9 scale, with 6 or greater considered acceptable) and percent disease (0-100%) data were collected weekly and percent green cover was collected monthly using a light box.

In year one, rolling decreased disease activity in the absence of other treatments. When Civitas One was included or Sulfur DF+PK Plus was used < 1.5% disease was observed regardless of rolling treatments. In year two, Civitas One with any fertility or rolling treatment, as well as Sulfur DF+PK Plus with or without rolling, and Sulfur DF with rolling resulted in <1.5% Microdochium patch incidence. Treatments including Civitas One without rolling produced the highest turf quality; however, Civitas One with rolling resulted in the lowest turf quality due to abiotic damage.

Introduction:

The cultural practice of rolling has been shown to influence disease incidence on turfgrass. Inguagiato (2009) reported a 5 to 6% decrease in anthracnose under moderate disease pressure on an annual bluegrass (*Poa annua* L.) putting green when rolling every other day and Giordano (2012) observed that rolling a mixed stand of annual bluegrass and creeping bentgrass (*Agrostis stolonifera* L.) 5 days a week reduced AUDPC values for dollar spot (*Sclerotinia homeocarpa*) incidence with an even greater decrease in AUDPC values when rolling occurred 2 times a day. In Corvallis, preliminary trials showed rolling was able to reduce Microdochium patch by as much as 75% on an annual bluegrass putting green (Mattox et al., 2014). Researchers suspect a correlation to this cultural practice and changes in the soil microbial community of a sand-based putting green. Giordano (2013) revealed an increase in bacterial populations and a decrease in fungal populations within rolled plots using phospholipid fatty acid analysis. This decrease in fungal populations may explain the decrease in dollar spot activity on creeping bentgrass greens observed by Giordano (2013). While preliminary research has been positive, further work is required to fully understand the effects of this cultural practice on Microdochium patch activity within annual bluegrass. In the search for alterative control methods it would also be advantageous to explore rolling combined with other traditional fungicide control options, such as sulfur, mineral oil and phosphite.

Sulfur has been used in agriculture for pest control for well over 2000 years and was recognized specifically for its ability to inhibit rust in wheat by the ancient Greeks (Beckerman, 2008; Smith and Secoy, 1975). Sulfur has more recently been shown to suppress Microdochium patch on Colonial bentgrass (*Agrostis tenuis*) putting greens when applied annually at 224 Kg ha^{-1} (Brauen et al., 1975). It is likely that soil acidity is affected by sulfur additions as sulfur is oxidized by soil microorganisms to sulfuric acid that upon dissociation releases hydrogen ions (McCarty et al., 2003). A decrease in disease incidence may be the result as lower soil pH is considered to be less conducive to *M. nivale* infection (Couch, 1995; Smiley et al., 1992; Smith, 1958). Other possibilities of disease inhibition by sulfur include; respiratory inhibition in fungal mitochondria (Williams and Cooper, 2004), direct fungal toxicity due to sulfur reduction to hydrogen sulfide (Tweedy, 1981), or increased oxidation of the plant antioxidant glutathione (Beffa, 1993; Williams and Cooper, 2004; Bloem et al., 2005). In addition to sulfur providing benefits to disease inhibition, it is also an essential element for turfgrass growth and is required for some secondary metabolites involved in pathogen resistance (Bloem et al., 2005; Goss, 1984). Elemental sulfur products are registered as organic by the Organic

Materials Review Institute (OMRI, 2015b) and are likely candidates for alternatives to traditional fungicides regarding Microdochium patch, although have also been associated with a greater risk of anthracnose (Kowalewski et al., 2015). While sulfur has been shown to reduce Microdochium patch activity on colonial bentgrass, work on other species such as annual bluegrass, the dominant turf on putting greens in the Pacific Northwest (Lyman et al., 2007), is limited.

Civitas One is an OMRI (2015a) registered turfgrass fungicide that consists of a food-grade emulsifier and synthetic isoparaffin (Hsiang et al., 2013) in addition to the green pigment Harmonizer containing polychlorinated copper (Cu) II phthalocyanine (Nash, 2011). Civitas and Harmonizer have been shown to suppress *M. nivale* when tested in vitro (Nash, 2011) and greenhouse studies have shown that when applied to the soil, Civitas at 10 and 20% concentration suppressed Microdochium patch between 50 and 65% respectively on creeping bentgrass (Cortes-Barco et al. 2010a). Disease control through soil-based applications provide evidence that Civitas is influencing either the soil or soil microorganisms that leads to an induced systemic response in turfgrass (Cortes-Barco et al., 2010a and 2010b). Nash (2011) concluded similar results regarding experiments using a 5% solution of Harmonizer applied to the soil that resulted in a 32% suppression of dollar spot (*S. homeocarpa*) on creeping bentgrass.

Other field studies have shown that Civitas + Harmonizer provide control of anthracnose (*Colletotrichum cereale*) (Aynardi, 2011b; Popko and Jung, 2012) on a mixed creeping bentgrass and annual bluegrass putting green. Civitas alone provided control of drechslera leaf spot/melting out (*Drechslera poae*) on Kentucky bluegrass (*Poa pratensis*) (Uddin et al., 2010) and dollar spot on a mixed stand of creeping bentgrass / annual bluegrass (Aynardi et al., 2012b) and Gray leaf spot (*Pyricularia grisea*) on perennial ryegrass in Pennsylvania (*Lolium perenne*) (Aynardi et al., 2012a). However, Civitas in combination with Harmonizer did not control gray leaf spot on perennial ryegrass in Virginia (McCall and Focht, 2010), dollar spot on creeping bentgrass in North Carolina (Soika et al., 2011) or in Oklahoma (Smith and Walker, 2012) or Drechslera leaf spot / melting out on Kentucky bluegrass (Aynardi et al., 2011a). While multiple studies have been performed on a variety of turfgrass diseases and species, work concerning Microdochium patch on annual bluegrass is limited.

In turfgrass management, phosphite containing products are often marketed as fertilizers although they possesses fungicide characteristics (Vincelli and Dixon, 2005). They are products of phosphorus acid and are an active ingredient of phosphonate fungicides (Guest and Grant, 1991) that are considered to be a low-risk pesticide (Latin, 2011). Phosphonates are known to possess suppressive qualities against *Phytopthora* in a variety of crops (Fenn and Coffey, 1984), *Pythium* on creeping bentgrass and perennial ryegrass (Sanders et al., 1983; Cook et al., 2009), and have more recently been shown to suppress *M. nivale* in vitro (Dempsey et al., 2014) and in vivo on creeping bentgrass (Dempsey and Owen, 2010), velvet bentgrass (Dempsey et al., 2012) and annual bluegrass (Golembiewski and McDonald, 2011a; Dempsey et al., 2012). The mode of action of phosphite against plant pathogens has been linked to inducing defenses in the host or through direct mycelial inhibition (Fenn and Coffey, 1984; Grant et al., 1990; Jackson et al., 2000) and are one of the few truly systemic fungicides with translocation occurring in both the xylem and the phloem (Groussol et al., 1986; Ouimette and Coffey, 1990; Guest and Grant, 1991). While the effects of phosphite on Microdochium patch within annual bluegrass have been documented, research was limited and control was not equivalent to traditional fungicides.

As previously stated it would be advantageous to explore combinations of various alternatives to traditional fungicides in search for additive effects. Therefore, the objectives of this study were to quantify the effects of rolling, mineral oil and fertility products (sulfur and phosphite) on the incidence of Microdochium patch on an annual bluegrass putting green.

Methods and Materials:

Experimental Area Construction

A field study was conducted at Oregon State University's Lewis-Brown Horticulture Farm in Corvallis, OR from September 26, 2013 to April 12, 2014 and again from September 22, 2014 to April 12, 2015. The putting green used for this research was constructed in April 2013 by placing 15 cm of USGA specification (USGA, 1993) sand directly on the native McBee silty clay loam (UC Davis, 2015), which was graded to a 2% slope. Annual bluegrass was established by collecting 13mm diameter by 76mm depth aerification cores from an annual bluegrass putting green at the Corvallis Country Club in Corvallis, OR. Cores were spread using a Meter-Matic topdresser (Turfco, NE Blaine, MN).

Experimental Design

Experimental design was a 2 by 2 by 4 by 2 randomized complete split-plot design with four replications. Individual plots were 1.5 m^2 plots and the total experiment area was 96 m^2. Factors included year (one and two), rolling (whole-plot), fertility (split-plot) and mineral oil (split-plot). All rolling, fertility and mineral oil applications were made from September 26, 2013 to June 13, 2014 and again from September 22, 2014 to June 12, 2015. Rolling was applied five days a week using a 122cm golf course putting green roller (Tru-turf, Queensland, Australia). Fertility applications included Sulfur DF 12.22 Kg S ha^{-1}, PK Plus 3.66 Kg H_3PO_3 ha^{-1} and a combination of Sulfur DF and PK Plus applied every two weeks in addition to an untreated control. Mineral oil treatments were Civitas One 19.94 Kg a.i. ha^{-1} applied every two weeks compared to a control. Fertility and mineral oil treatments were applied with a CO_2-powered backpack sprayer with a 4 nozzle hand-held boom equipped with XR11015 nozzles using a pressure of 2.8 bars at the boom. Carrier volume was 814 L ha^{-1} and displacement speed was calibrated with a metronome.

Turfgrass Maintenance

The putting green was mowed as needed at a mowing height of 3.8 mm and clippings were removed. Annual nitrogen rates from April 2013 to March 2014 was 283 Kg N ha^{-1} and from April 2014 to March 2015 was 222 Kg N ha^{-1}. During the trial period, nitrogen applications were in the form of Urea (46N-0P-0K) and did not exceed 9.7 Kg N ha^{-1} every two weeks. Irrigation was applied as needed throughout the year; peak rates during summer heat stress were 2.54 cm per week. Topdressing was applied every two weeks from July 5, 2013 to September 05, 2013 and again from July 3, 2014 to September 02, 2014. Hollow-tine aerification using 13mm diameter tines on 5.1cm by 5.1cm spacing at a 76mm depth was performed on September 18, 2013, and again on June 19 and September 16, 2014. No fungicides were applied at any time throughout the study.

Response Variables

Response variables included percent disease, area under disease progress curve (AUDPC), turfgrass quality and percent green cover. Percent disease was a visual rating ranging from 0% to 100% collected weekly from September 27, 2013 to June 13, 2014 and October 2, 2014 to June 12, 2015. In order to compare the effects of the treatments during the period when the disease incidence was at its highest level (Figure 1.1), the peak of disease was determined for both years and then percent disease observed on these dates only (March 13, 2014 and February 16, 2015) was presented. To quantify disease development over the course of the experiments, area under disease progress curve (AUDPC) data was determined by taking the sum (Σ) of the average disease severity $[(y^i + y^{i+1})/2]$ between two observations (y^i) multiplied by the time interval between the observations $[t^{i+1}-t^i]$ using the formula $\Sigma[(y^i + y^{i+1})/2][t^{i+1}-t^i]$ as determined by Shaner and Finney (1977). AUDPC data for this trial was determined by using the percent disease data that was collected weekly from initial data collection (September 27, 2013 and October 02, 2014) up to the final data collection date (June 13, 2014 and June 12, 2015).

Turfgrass quality ratings were assigned using the NTEP rating system (Morris and Shearman, 2015) at the peak of disease (March 13, 2014 and February 16, 2015). Turfgrass quality ratings were determined by the amalgamation of five influences; color, density, uniformity, texture and damage due to stress or disease. Turfgrass quality rating were 1-9 with a 1 rating given to dead turf, 9 given to ideal turf and a rating of 6 being considered acceptable for putting green turf (Morris and Shearman, 2015).

Percent green cover was determined by collecting digital images using a Sony DSC-H9 Camera (Sony, Tokyo, Japan) attached to a 50.8cm wide by 61cm long (total area 0.31m^2) light box with four 40-Watt spring bulb lamps (TPC, Lighthouse Supply, Bristol, VA). The collected images were then analyzed using Sigma Scan (v.5.0, SPSS, Inc. Chicago, IL) in order to determine percent green cover (Richardson et al., 2001). The threshold for the digital photo analysis was set to Hue 45-255 and Saturation 30-100.

Statistical Analysis

Data were subjected to analysis of variance using SAS 9.2 Proc Mixed (SAS Institute Inc., Cary, N.C.). Factors within this analysis included year, rolling, fertility and mineral oil. When main effects and interactions were significant Fisher's Protected Least Significant Difference (LSD) was used to separate individual means at a 0.05 level of probability. Initial analysis of variance observed significant year or interactions between year and the remaining factors for all of the response variables; therefore, data were analyzed and presented separately by year.

Results

Percent Disease

In year one, at the peak of disease incidence, interactions between rolling by mineral oil, rolling by fertility, and mineral oil by fertility on percent disease cover were observed (Table 1.1). Regarding the rolling by mineral oil interaction, Civitas One with or without rolling produced the lowest percent disease (<1%), followed by rolling without Civitas and finally the control (no rolling or Civitas) (Figure 1.2).

In reference to rolling by fertility interactions, Sulfur DF+PK Plus regardless of rolling treatment provided the best disease control with Microdochium patch levels of less than 0.5% at the peak of disease (Figure 1.3). Sulfur DF with or without rolling and PK Plus with rolling provided ≤ 5%, followed by PK Plus without rolling (8%). The rolling treatment without fertility was able to reduce Microdochium patch incidence (26%) compared to plots without rolling or fertility treatments (37%).

Mineral oil by fertility interactions including Civitas One without fertility, Civitas One with Sulfur DF or PK Plus as well as Sulfur DF+PK Plus with or without Civitas One resulted in the highest level of disease suppression with < 1.5 % respectively (Figure 1.4). The fertility treatments of Sulfur DF or PK Plus applied in the absence of Civitas One resulted in the following group of disease suppression with 8% and 11% disease respectively. In the absence of Civitas One or fertility treatments, 62% disease was observed.

At the peak of disease for the second year of the study, a three-way interaction between rolling, mineral oil and fertility was observed (Table 1.1).

Civitas One combined with all fertility treatments with and without rolling, as well as Sulfur DF+PK Plus with and without rolling and Sulfur DF with rolling resulted in the least amount of Microdochium patch (< 1.5%) (Figure 1.5). This group was followed by PK Plus treatments with rolling (5%), then by Sulfur DF without rolling (9%) and then by PK Plus without rolling (29%). Rolling alone (without fertilizer or mineral oil) and finally the control (no rolling, fertility or mineral oil) resulted in the poorest control with 30% and 53% disease respectively.

Area Under Disease Progress Curve

In year one, an interaction between mineral oil and fertility was observed (Table 1.1). The lowest AUDPC values occurred in treatments receiving Civitas One regardless of fertility treatment, as well as plots that received Sulfur, PK Plus or SulfurDF+PK Plus without Civitas (<9.8) (Figure 1.6). The highest AUDPC values were observed in treatments not receiving mineral oil or fertility, 55.4.

Year two AUDPC results identified a three-way interaction between rolling, fertility and mineral oil (Table 1.1). Civitas One with or without rolling and all fertility treatments combinations, Sulfur DF+PK Plus with and without rolling, and rolling with Sulfur DF treatments resulted in the lowest AUDPC values (<1.8) (Figure 1.7). This group was followed by treatments combining rolling and PK Plus, as well as Sulfur DF in the absence of rolling (8.0 to 8.5). PK plus without rolling and plots only receiving the rolling treatment, were in the next group (34 to 39). In the absence of Civitas One, fertility or rolling treatments, the largest AUDPC values were observed, 58.6.

Turfgrass Quality

In year one, turfgrass quality results indicated a significant interaction between the rolling by mineral oil treatments as well as mineral oil by fertility treatments (Table 1.1). The highest turfgrass quality was observed in plots without rolling and treated with Civitas One (7.5) (Figure 1.8). The following group included plots with rolling and Civitas One treatments as well as plots with rolling not treated with Civitas One, with ratings of 5.4 and 4.8, respectively. The lowest turfgrass quality ratings were observed in plots not receiving rolling or mineral oil treatments (4.5).

Regarding the effects of mineral oil by fertility interactions on turf quality, the highest turfgrass quality was observed in plots receiving Civitas One with Sulfur DF, PK Plus, or Sulfur DF+PK Plus (6.9 to 6.2 respectively) (Figure 1.9). This group was followed by Civitas One in the absence of fertility (5.9), and Sulfur DF+PK Plus in the absence of Civitas One (5.6). The next group included either Sulfur DF or PK Plus in the absence of Civitas One with ratings of 4.9 and 4.8 respectively. The lowest rating was observed in the control, the absence of mineral oil or fertility treatments (3.3).

Year two turfgrass quality analysis resulted in a significant interaction between rolling by mineral oil treatments and mineral oil by fertility treatments (Table 1.1). The highest turfgrass quality was observed in plots without rolling and receiving treatments including Civitas One (6.8) followed by plots with rolling in the absence of Civitas One (5.0), followed by plots without rolling in the absence of

Civitas One (4.3) (Figure 1.10). Plots with rolling in combination with Civitas One resulted in the lowest turfgrass quality ratings (2.4).

In reference to the fertility by mineral interactions, the highest turfgrass quality was observed in plots receiving Sulfur DF+PK Plus treatments in the absence of Civitas One (6.2) (Figure 1.11). This treatment was followed by Sulfur DF+PK Plus with Civitas One, Civitas One without fertility, and PK Plus or Sulfur DF with or without Civitas, ranging 4.3 to 4.9. The lowest rating was observed in the absence of mineral oil or fertility treatments (3.3).

Percent Green Cover

Year one percent green cover resulted in a significant interaction between rolling by mineral oil treatments and mineral oil by fertility treatments (Table 1.1). Regarding the rolling by mineral oil interaction, the highest percent green cover was observed in plots without rolling and receiving Civitas One treatments (99.8%), followed by plots with rolling that received Civitas One (96.0%) (Figure 1.12). Treatments not receiving Civitas One with or without rolling produced the lowest percent green cover (88%).

When the mineral oil by fertility interaction is considered, the highest percent green cover was observed in all plots receiving Civitas One with or without fertility, as well as Sulfur DF+PK Plus without Civitas One (> 97%), followed by Sulfur DF or PK plus treatments in the absence of Civitas One with both treatments proving 93% green cover (Figure 1.13). The lowest percent green cover was observed in the control, where plots did not receive mineral oil or fertility treatments (68%).

Year two Percent Green Cover resulted in significant rolling by mineral oil, and rolling by fertility, and mineral oil by fertility interactions (Table 1.1). Regarding the rolling and mineral oil interaction, the highest percent green cover was observed plots without rolling and receiving Civitas One treatments (99.9%) and plots with rolling in the absence of Civitas One (95.9%) (Figure 1.14). These treatments were followed by plots without rolling in the absence of Civitas One (82.1%), and finally plots with rolling combined with Civitas One, which provided the lowest percent green cover, 72.2%.

When exploring the rolling by fertility interaction, the highest percent green cover was observed in plots without rolling and receiving the Sulfur DF+PK Plus (99.6%) as well as plots without rolling and receiving Sulfur DF (96.0%) (Figure 1.15). The following group included PK Plus with or without rolling and plots with rolling in the absence of fertility, 92.3%, 89.1% and 88.2% green cover respectively. The lowest percent green cover was observed in plots without rolling in the absence of fertility treatments as well as in plots with rolling and Sulfur DF and with rolling and Sulfur DF+PK Plus resulting in 79.2%, 78.3% and 77.4% green cover, respectively.

Finally, in reference to the mineral oil by fertility interaction, the highest percent green cover was observed in plots receiving Sulfur DF+PK Plus in the absence of Civitas One (99.4%), followed by Sulfur DF in the absence of Civitas One (94.8%), Civitas One with PK Plus (93.5%) and Civitas One without fertility (93.4%)

(Figure 1.16). These treatments were followed by PK Plus without Civitas One (87.9%), then Civitas One in combination with Sulfur DF and Civitas One in combination with Sulfur DF+PK Plus (79.5 to 77.6%). The lowest percent green cover was observed in the control plots, no mineral oil or fertility treatments (73.9%).

Discussion

Rolling as a cultural practice did provide some level of M. patch control in the absence of fertility or mineral oil treatments. Rolling has been shown to reduce other turfgrass diseases (Inguagiato, et al., 2009; Giordano et al., 2012) in particular Microdochium patch (Mattox et al., 2014). This is in contrast to observations by Nikolai (2001) on creeping bentgrass where Microdochium patch incidence was greater on rolled plots. Microdochium patch was observed three weeks after rolling treatments began and only in the second year of Nikolai's study. It could be that the benefits of rolling had not yet had an effect on disease suppression. Giordano (2013) speculated that the effect of rolling on microbial populations in the soil might be the cause of disease control and it is likely that on sand putting greens the impact of rolling on microbial characteristics would take longer than three weeks to occur.

When Civitas One was applied, Microdochium patch suppression of 98% and 99% was observed for both years respectively. Civitas One is comprised of the mineral oil Civitas and the green pigment Harmonizer, both of which have been shown separately to suppress turfgrass diseases. Civitas has previously been shown to suppress *Microdochium nivale* in the greenhouse on creeping bentgrass between

50 and 65% when applied to the soil at 10 and 20% concentrations (Cortes-Barco et al., 2010a) and Harmonizer applied to the soil as a 5% solution has been shown to suppress Dollar Spot on creeping bentgrass by 32% in the greenhouse (Nash, 2011).

Studies comparing Civitas + Harmonizer to tradition fungicides have observed control equivalent to many traditional fungicides. This is true concerning anthracnose (Aynardi et al., 2011b; Popko and Jung, 2012) and dollar spot (Aynardi et al., 2012b) on a mixed creeping bentgrass / annual bluegrass putting green, drechslera leaf spot/melting out on Kentucky bluegrass (Uddin et al., 2010) and Gray leaf spot (*Pyricularia grisea*) on perennial ryegrass (*Lolium perenne*) (Aynardi et al., 2012a). This is the first report of Civitas One suppressing Microdochium patch on annual bluegrass putting greens.

In the absence of mineral oil, Sulfur DF combined with PK Plus provided less than 1% Microdochium patch. These results suggest an additive effect of this fertility treatment combination concerning Microdochium patch control. While Microdochium patch control has previously been reported through the use of sulfur on colonial bentgrass at an annual rate of 224 Kg ha^{-1} (Brauen et al., 1975) and on multiple turfgrass species including annual bluegrass using potassium phosphites at rates of 3.66 to 3.86 Kg ha^{-1} every two weeks (Golembiewski and McDonald, 2011a; and Dempsey et al. 2012), this is the first report of an additive effect of the product combination. This combination could simply be toxic to the pathogen (Tweedy, 1981; Williams and Cooper, 2004; Dempsey et al., 2014). However an alternative explanation is that sulfur is changing the soil acidity (McCarty, 2003), while potassium phosphite is inducing defenses in the host (Fenn and Coffey, 1984; Grant et al., 1990; Jackson et al., 2000). Even though sulfur has provided some Microdochium patch suppression, applications of sulfur have been associated with a greater risk of anthracnose (Kowalewski, et al., 2015) and is also reported to increase the risk of black layer (Berndt and Vargas, 2008).

Abiotic damage was observed in this study as a result of Civitas One treatments in combination with rolling. Research at Cornell University revealed similar chronic phytotoxicity on a field study taking place over two consecutive summers using Civitas and Harmonizer on a mixed stand of bentgrass and annual bluegrass putting greens (Kreuser, 2014). Kreuser (2014) suggested that the phytotoxicity was due to stomatal occlusion by the Civitas oil that resulted in reduced gas exchange.

Conclusion:

Rolling was able to reduce Microdochium patch intensity on annual bluegrass putting greens however, only in the absence of other fungicide alternative disease control techniques such as Civitas One, Sulfur DF or PK Plus treatments.

In spite of the intensity of M. patch disease observed in this study, suppression of M. patch through the use of alternative controls to levels acceptable for golf course putting greens was observed for multiple treatment combinations. Bi-weekly applications of the mineral oil Civitas One was able to reduce M. patch incidence to levels < 1.5 %. However, abiotic damage was observed when rolling was combined with Civitas One. Further research is warranted regarding application rates and timings in order to determine a safe use of this product for golf course putting green applications.

Sulfur DF combined with PK Plus suppressed disease activity to levels comparable to Civitas One, less than 1%. These results suggest an additive effect when these fertility treatments are applied in combination. In addition, abiotic damage was not observed in the Sulfur DF+PK Plus treatments, therefore this combination provides an IPM tool that would assist turfgrass managers in managing M. patch in the absence of traditional fungicides. Further research is warranted to better understand the mechanisms involved in the additive effect of M. patch control with Sulfur DF and PK Plus combinations.

Figures

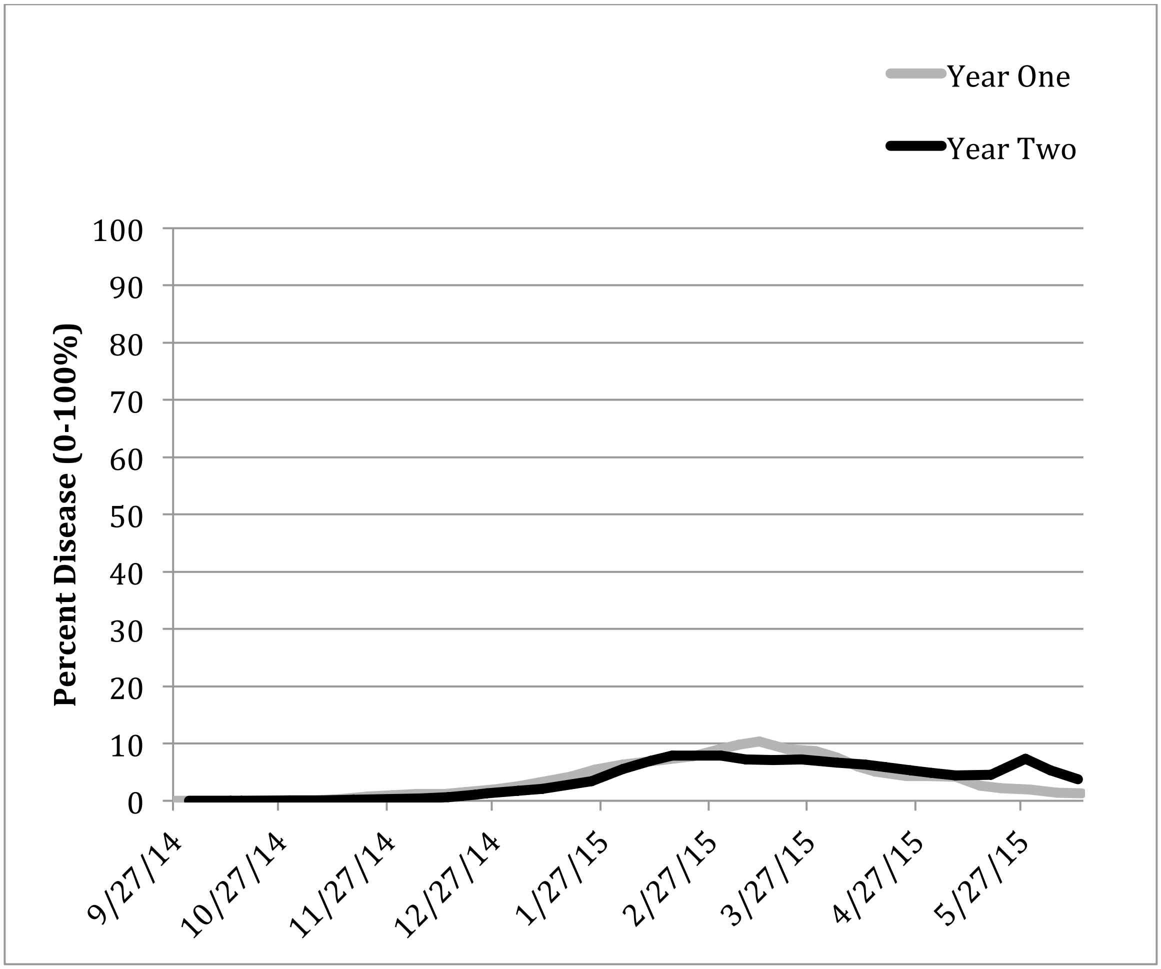

Figure 1.1: Microdochium patch development over time on an annual bluegrass putting green in Corvallis, OR over two years. Disease development over time measured as total combined disease on all plots for year one (26th of September 2013 to the 13th of June 2014) and year two (22nd of September 2014 to the 12th of June 2015) of a two-year experiment in Corvallis, OR.

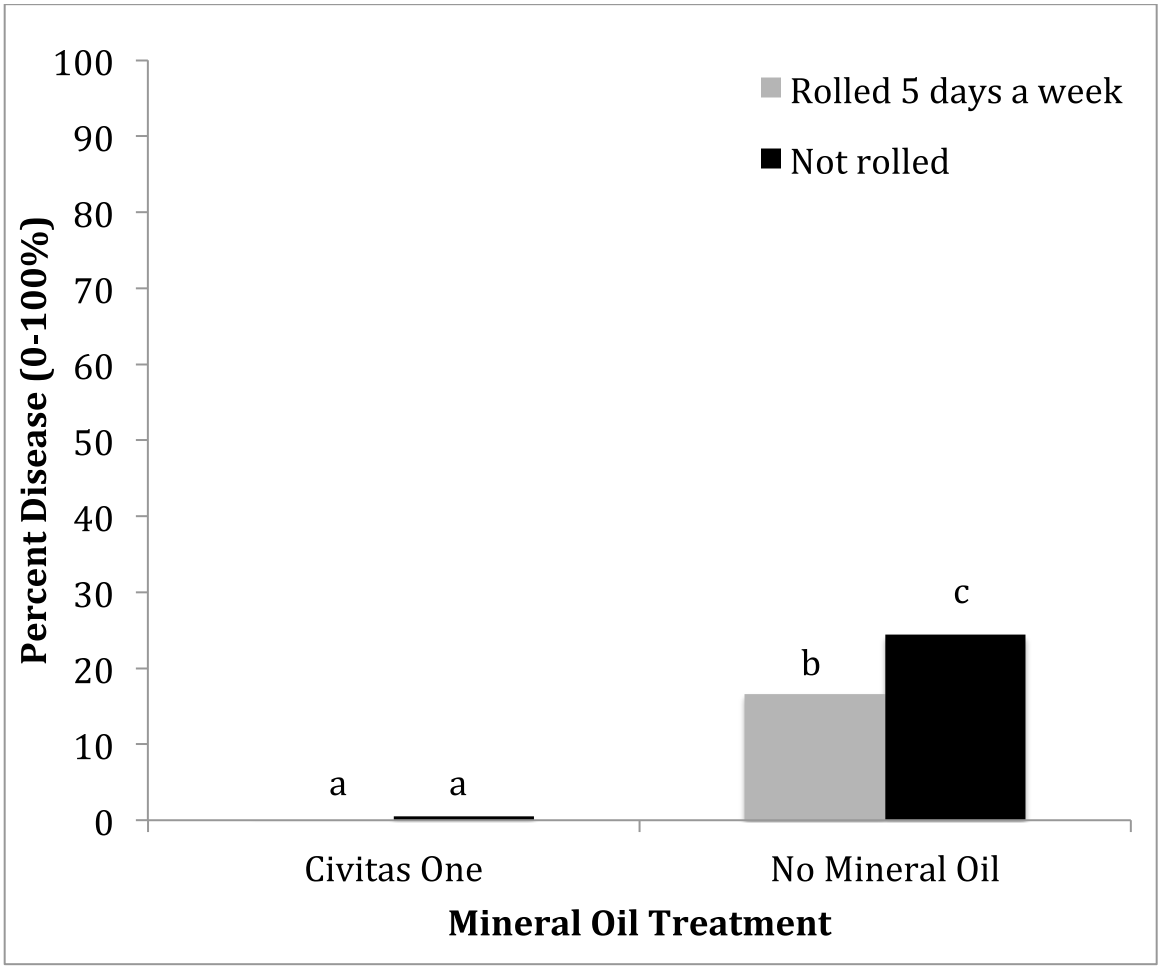

Figure 1.2: Effects of rolling and mineral oil treatments on percent disease on an annual bluegrass putting green in Corvallis, OR on March 13, 2014. Civitas One was applied every other week at 19.94 Kg a.i. ha^{-1}. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at $P \leq 0.05$. Means values represent 16 data points over 4 replications and 4 fertility treatments.

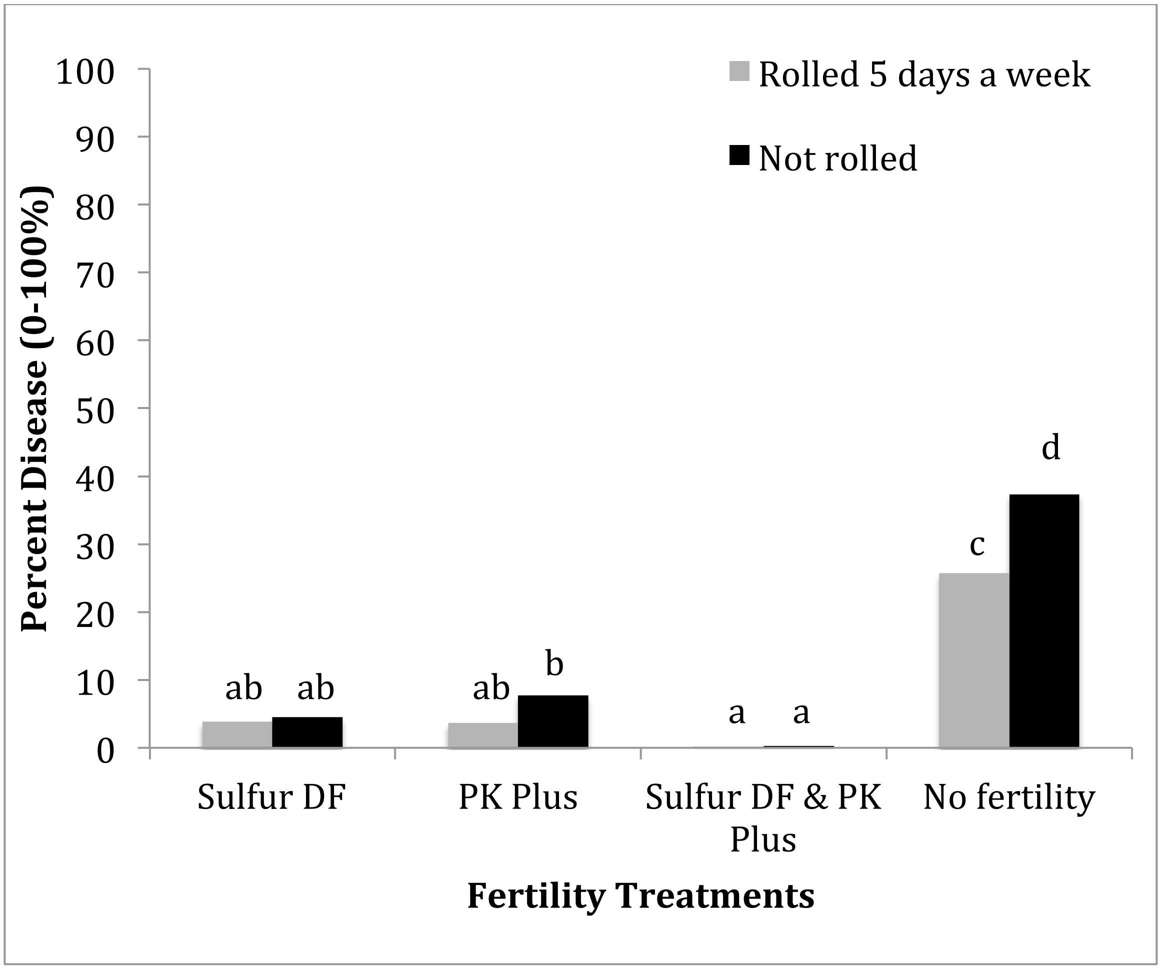

Figure 1.3: Effects of rolling and fertility treatments on percent disease on an annual bluegrass putting green in Corvallis, OR on March 13, 2014. Sulfur DF at 12.22 Kg S ha^{-1}, PK Plus at 3.66 Kg H_3PO_3 ha^{-1}, and Sulfur DF+PK Plus was applied every other week. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at P≤ 0.05. Means represent 8 data points over 4 replications and 2 mineral oil treatments.

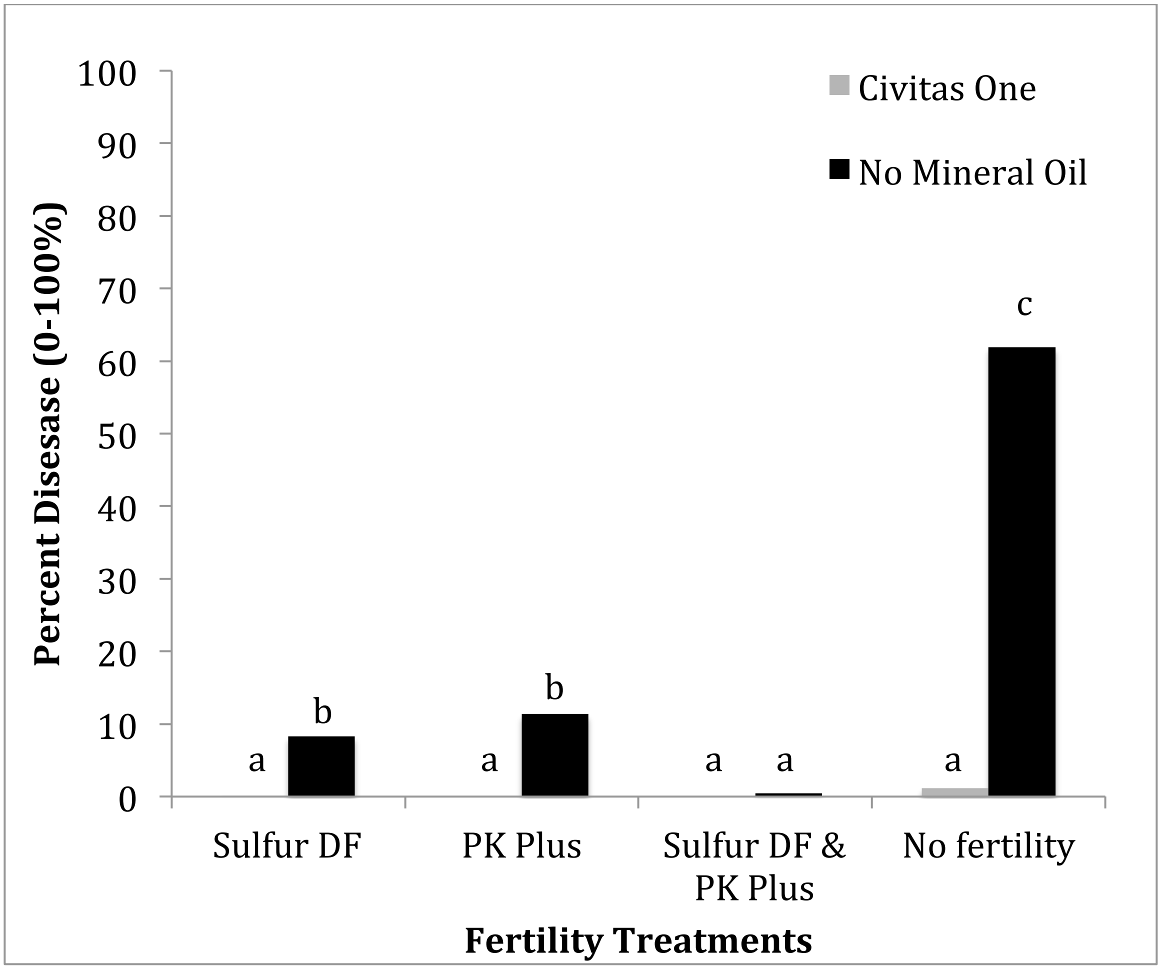

Figure 1.4: Effects of mineral oil and fertility treatments on percent disease on an annual bluegrass putting green in Corvallis, OR on March 13, 2014. Civitas One at 19.94 Kg a.i. ha^{-1}, Sulfur at DF 12.22 Kg S ha^{-1}, PK Plus at 3.66 Kg H_3PO_3 ha^{-1}, and Sulfur DF+PK was applied every other week. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at $P \leq 0.05$. Means represent 8 data points, across 4 replications and 2 rolling treatments.

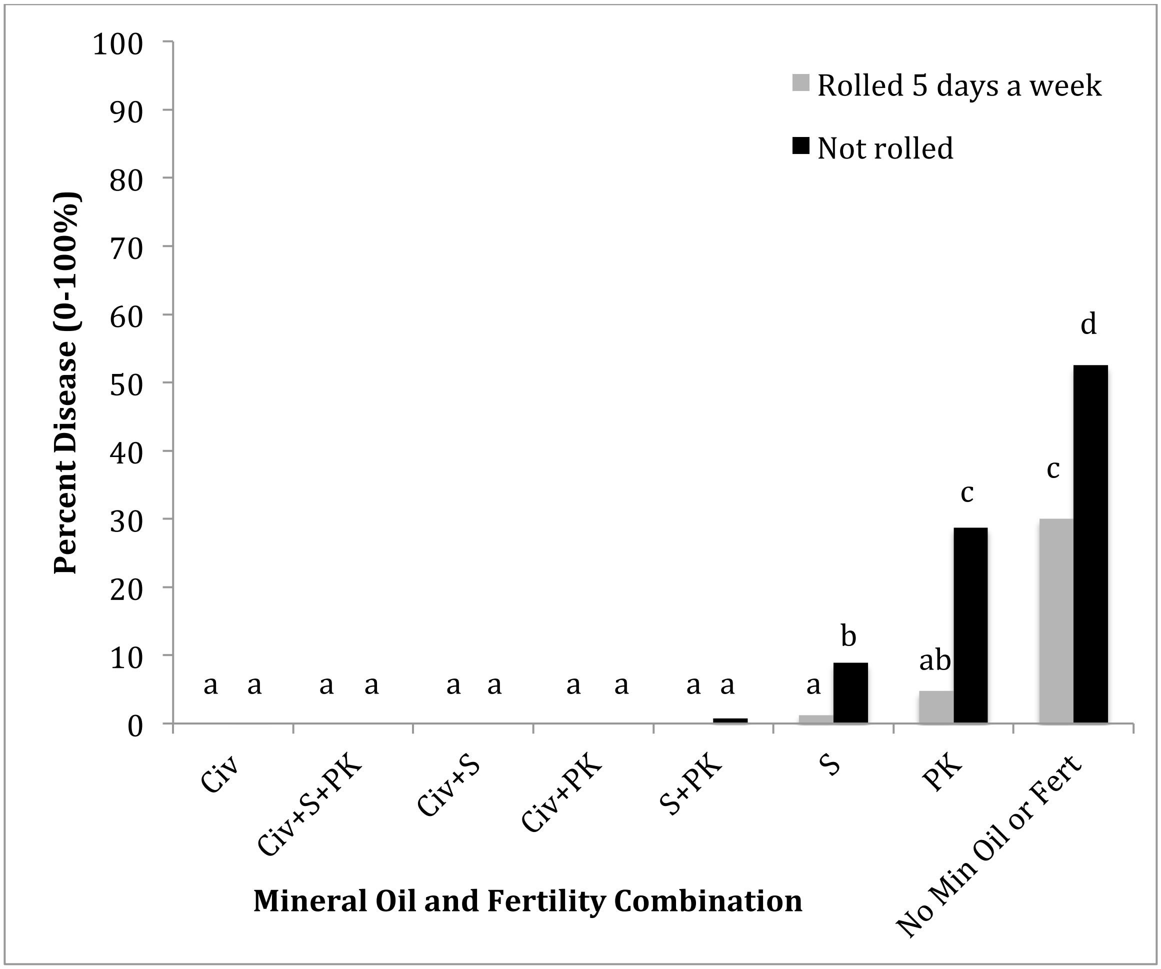

Figure 1.5: Effects of rolling, mineral oil (Min Oil) and fertility (Fert) treatments on percent disease on an annual bluegrass putting green in Corvallis, OR on February 16, 2015. Civitas One (Civ) at 19.94 Kg a.i. ha^{-1}, Sulfur DF (S) 12.22 Kg S ha^{-1}, PK Plus (PK) 3.66 Kg H_3PO_3 ha^{-1} and Sulfur DF+PK Plus was applied every other week. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at P≤ 0.05. Means represent 4 data points across 4 replications.

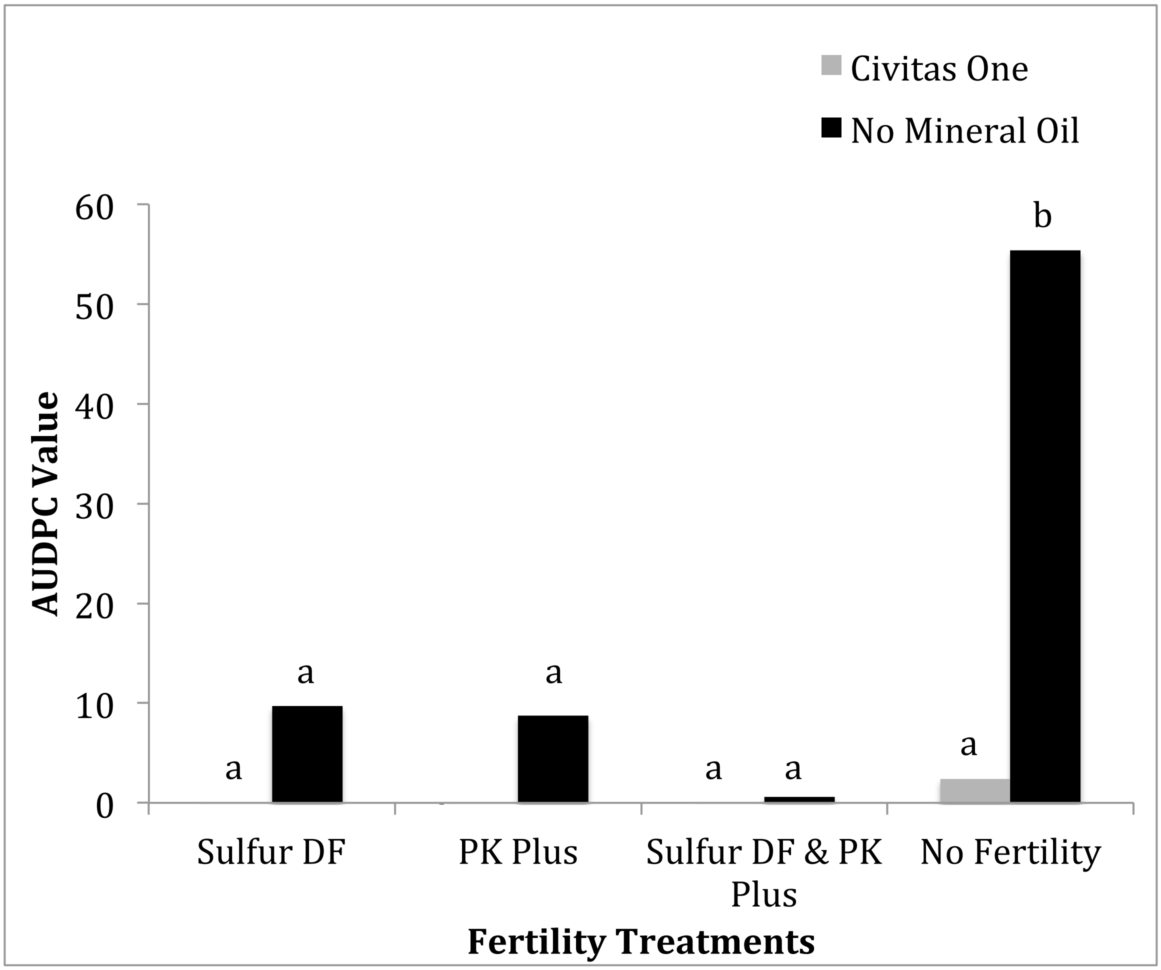

Figure 1.6: Effects of mineral oil and fertility treatments on area under disease progress curve values in Corvallis, OR from September 27, 2013 to June 13, 2014 on an annual bluegrass putting green. Civitas One 19.94 Kg a.i. ha^{-1}, Sulfur DF 12.22 Kg S ha^{-1}, PK Plus 3.66 Kg H_3PO_3 ha^{-1} and Sulfur DF+PK Plus were applied every other week. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at $P \leq 0.05$. Means represent 8 data points, across 4 replications and 2 rolling treatments.

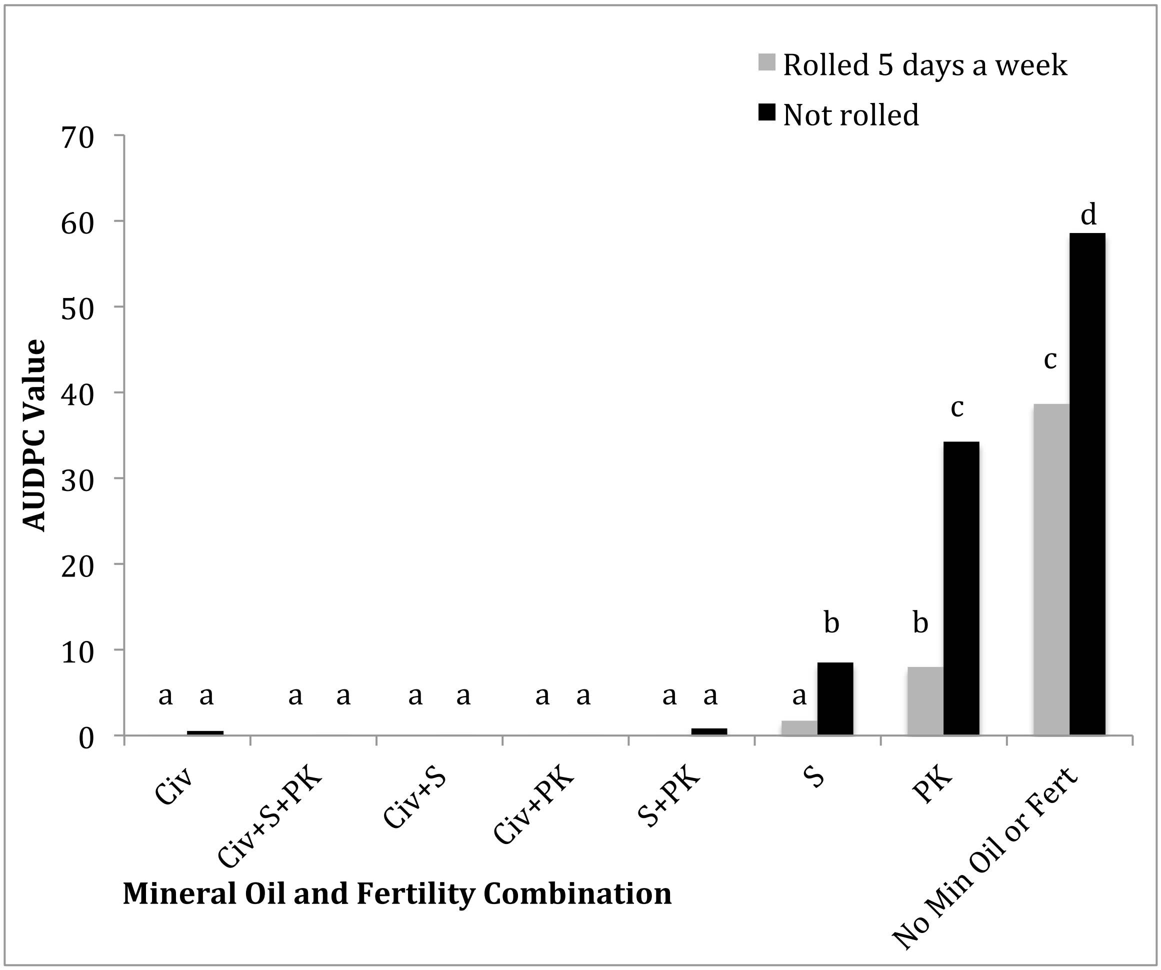

Figure 1.7: Effects of rolling, mineral oil and fertility treatments on area under disease progress curve values in Corvallis, OR from October 02, 2014 to June 12, 2015 on an annual bluegrass putting green. Civitas One 19.94 Kg a.i. ha^{-1}, Sulfur DF 12.22 Kg S ha^{-1}, PK Plus 3.66 Kg H_3PO_3 ha^{-1} and Sulfur DF+PK Plus were applied every other week. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at P≤ 0.05. Means represent 4 data points over 4 replications.

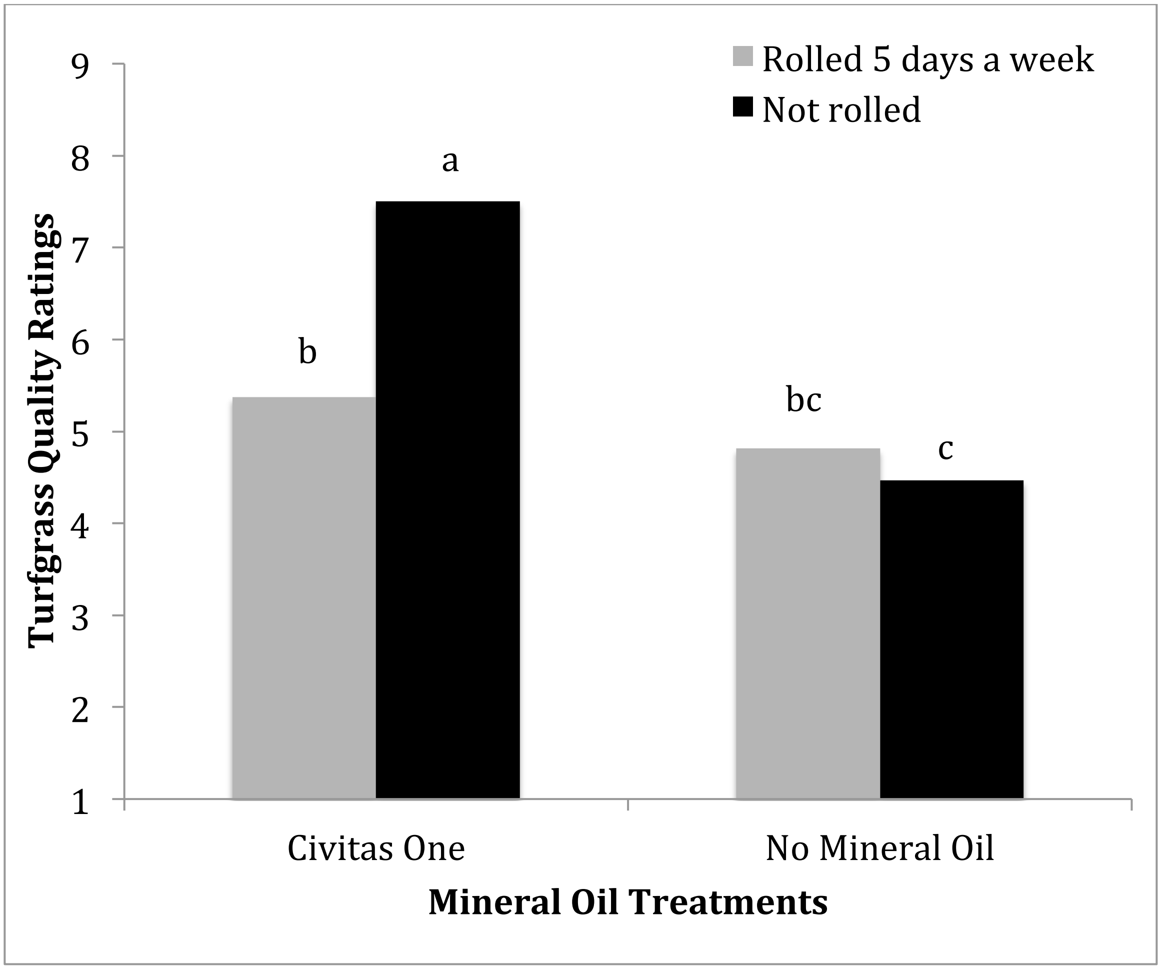

Figure 1.8: Effects of rolling and mineral oil treatments on turfgrass quality ratings on an annual bluegrass putting green in Corvallis, OR on March 13, 2014. Civitas One was applied every other week at 19.94 Kg a.i. ha^{-1}. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at $P \leq 0.05$. Means values represent 16 data points over 4 replications and 4 fertility treatments.

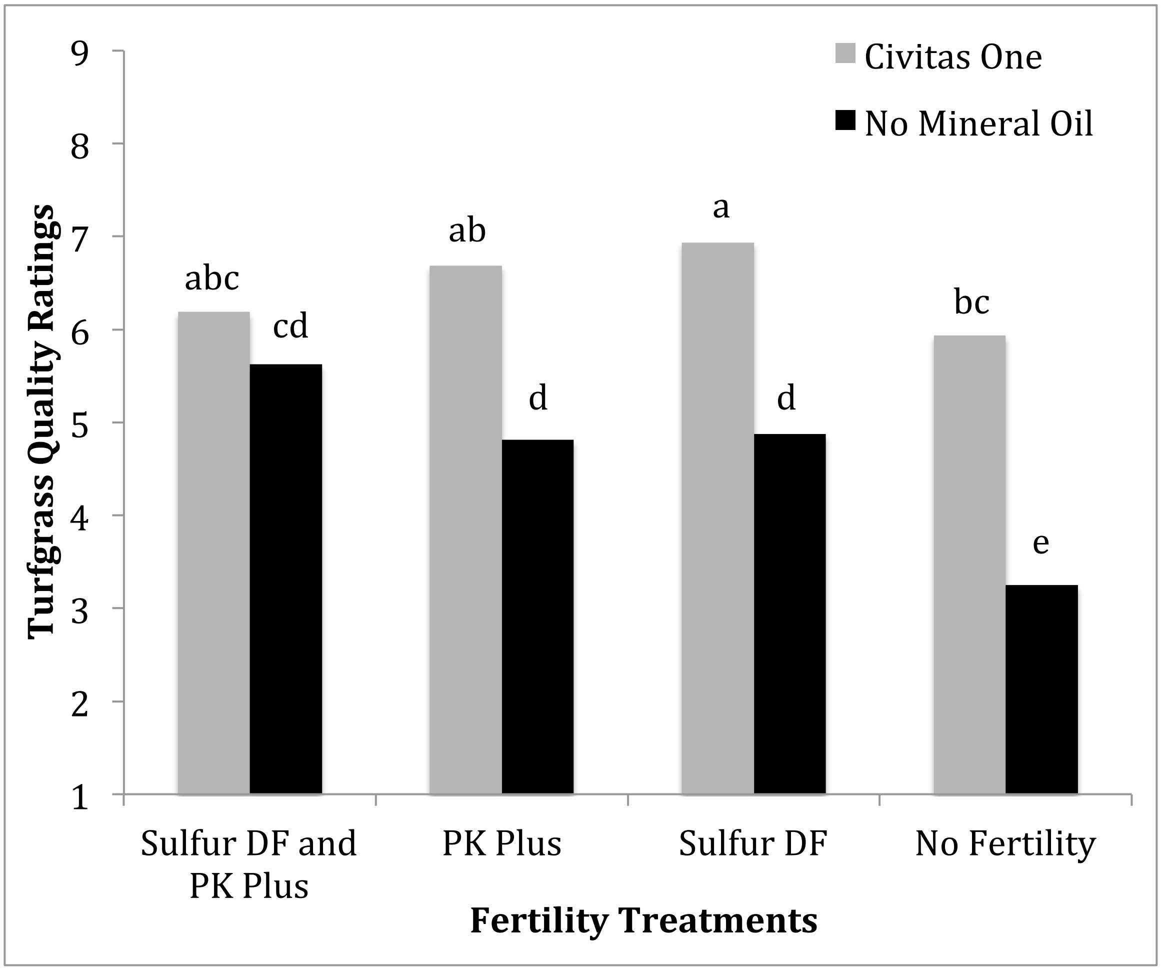

Figure 1.9: Effects of mineral oil and fertility treatments on turfgrass quality ratings in Corvallis, OR on an annual bluegrass putting green on March 13, 2014. Civitas One 19.94 Kg a.i. ha^{-1}, Sulfur DF 12.22 Kg S ha^{-1}, PK Plus 3.66 Kg H_3PO_3 ha^{-1} and Sulfur DF+PK Plus were applied every other week. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at $P \leq 0.05$. Means represent 8 data points, across 4 replications and 2 rolling treatments.

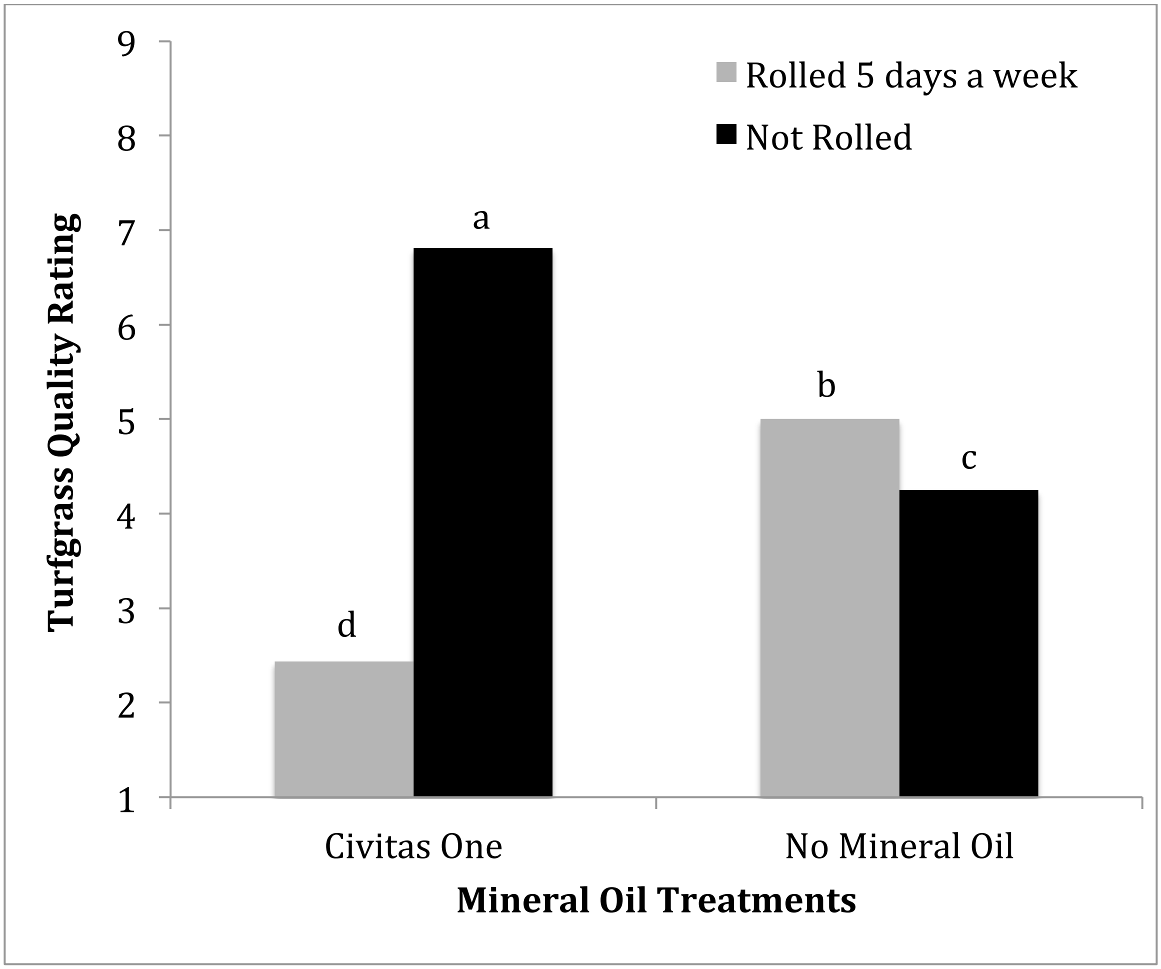

Figure 1.10: Effects of rolling and mineral oil treatments on turfgrass quality ratings on an annual bluegrass putting green in Corvallis, OR on February 16, 2015. Civitas One was applied every other week at 19.94 Kg a.i. ha^{-1}. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at P≤ 0.05. Means values represent 16 data points over 4 replications and 4 fertility treatments.

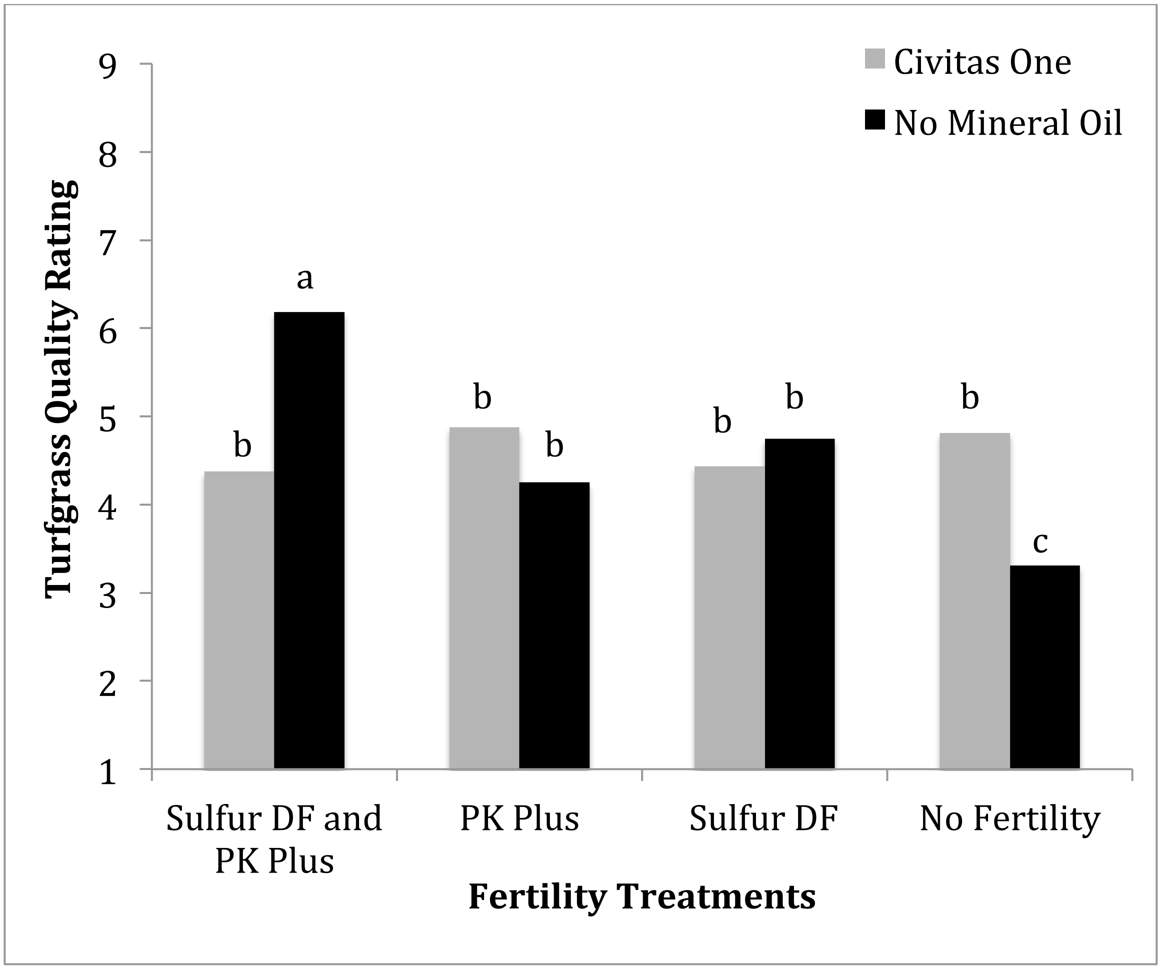

Figure 1.11: Effects of mineral oil and fertility treatments on turfgrass quality ratings in Corvallis, OR on an annual bluegrass putting green on February 16, 2015. Civitas One 19.94 Kg a.i. ha^{-1}, Sulfur DF 12.22 Kg S ha^{-1}, PK Plus 3.66 Kg H_3PO_3 ha^{-1} and Sulfur DF+PK Plus were applied every other week. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at P≤ 0.05. Means represent 8 data points, across 4 replications and 2 rolling treatments.

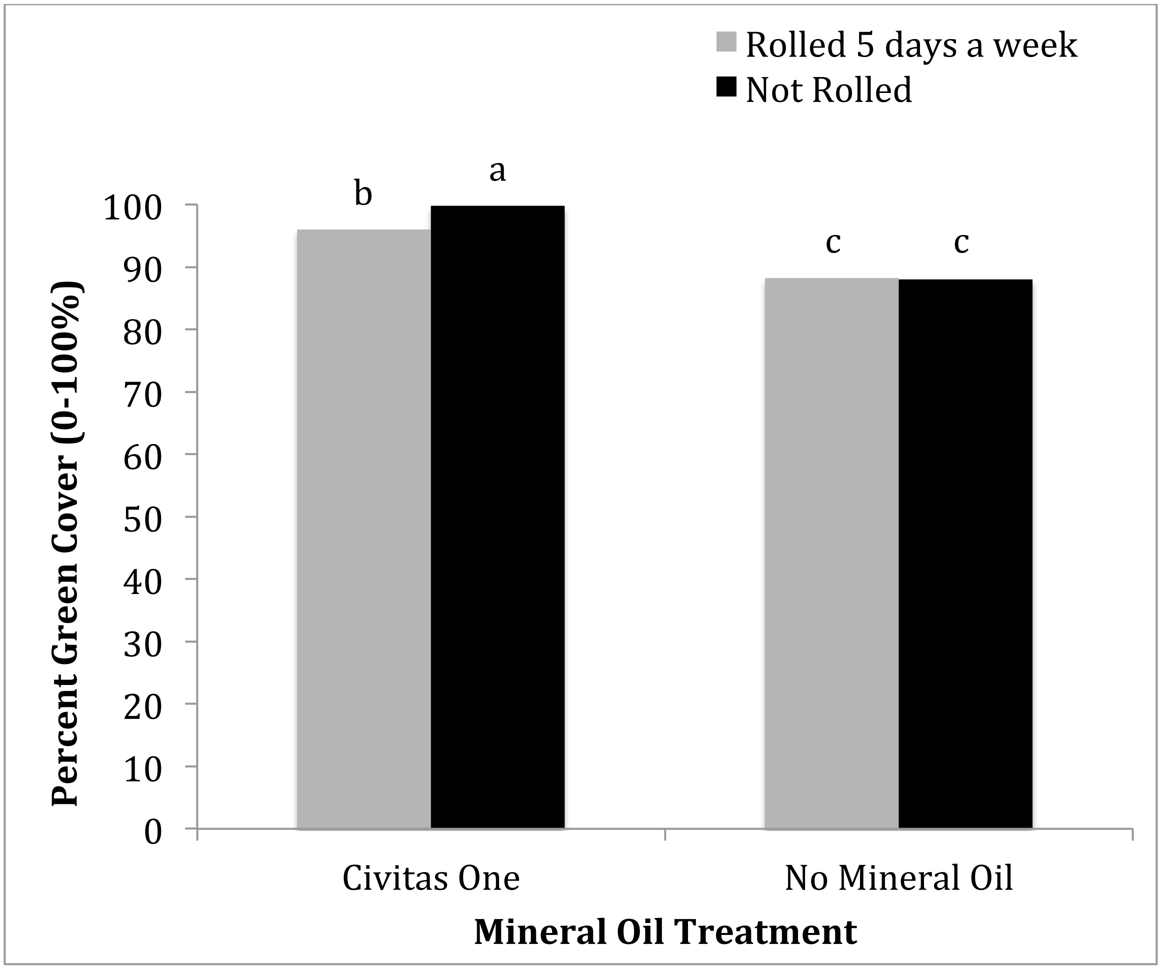

Figure 1.12: Effects of rolling and mineral oil treatments on percent green cover on an annual bluegrass putting green in Corvallis, OR on March 13, 2014. Civitas One was applied every other week at 19.94 Kg a.i. ha^{-1}. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at $P \leq 0.05$. Means values represent 16 data points over 4 replications and 4 fertility treatments.

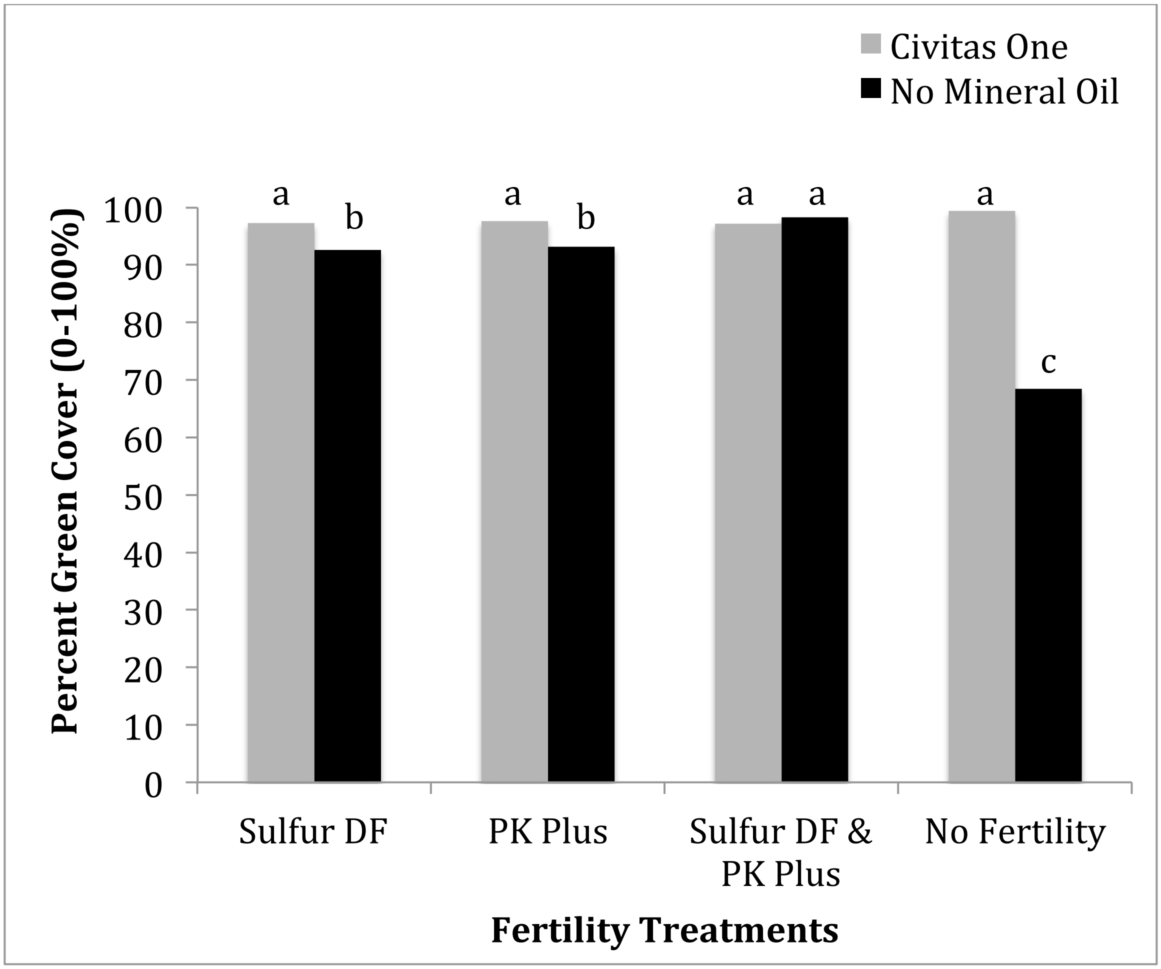

Figure 1.13: Effects of mineral oil and fertility treatments on percent green cover in Corvallis, OR on an annual bluegrass putting green on March 13, 2014. Civitas One 19.94 Kg a.i. ha^{-1}, Sulfur DF 12.22 Kg S ha^{-1}, PK Plus 3.66 Kg H_3PO_3 ha^{-1} and Sulfur DF+PK Plus were applied every other week. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at P≤ 0.05. Means represent 8 data points, across 4 replications and 2 rolling treatments.

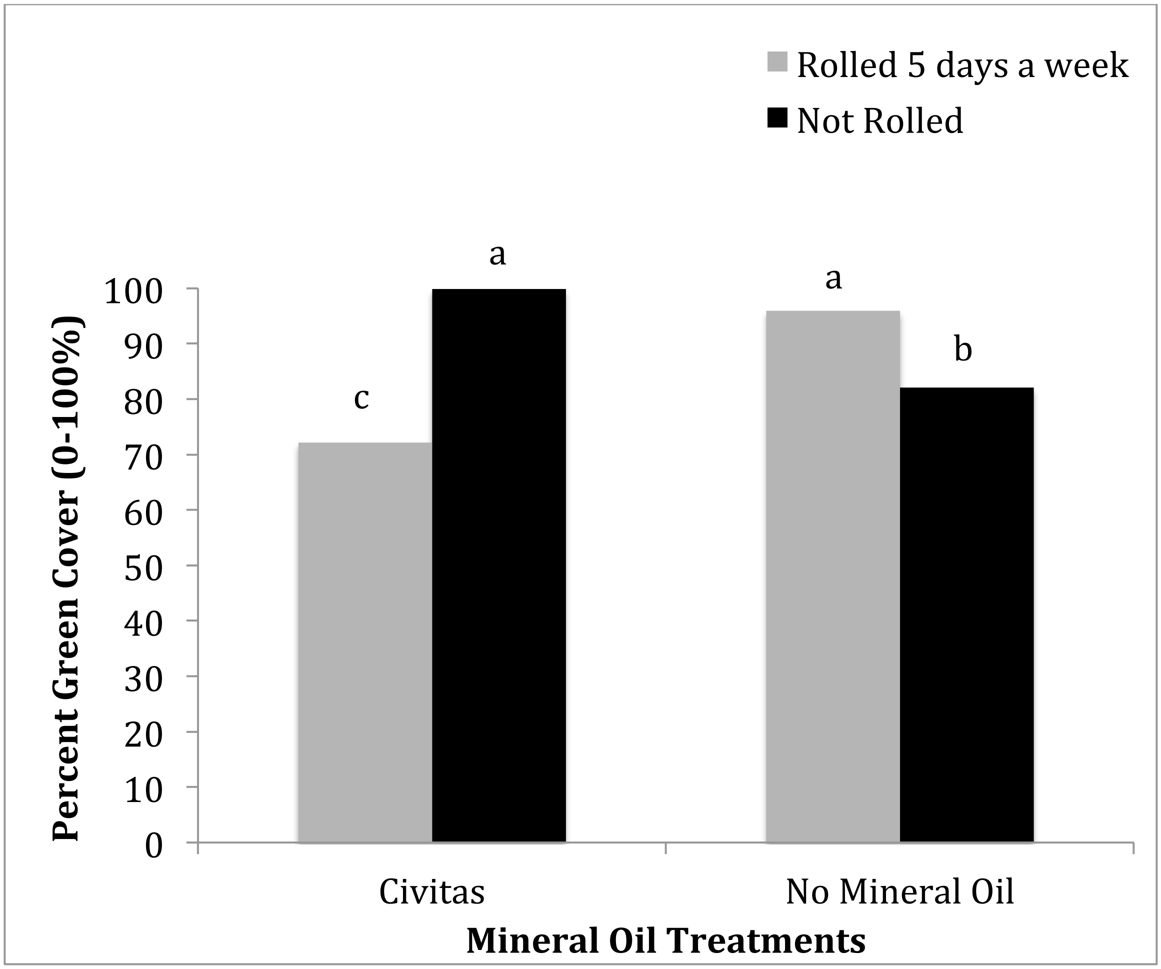

Figure 1.14: Effects of rolling and mineral oil treatments on percent green cover on an annual bluegrass putting green in Corvallis, OR on February 16, 2015. Civitas One was applied every other week at 19.94 Kg a.i. ha^{-1}. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at $P \leq 0.05$. Means values represent 16 data points over 4 replications and 4 fertility treatments.

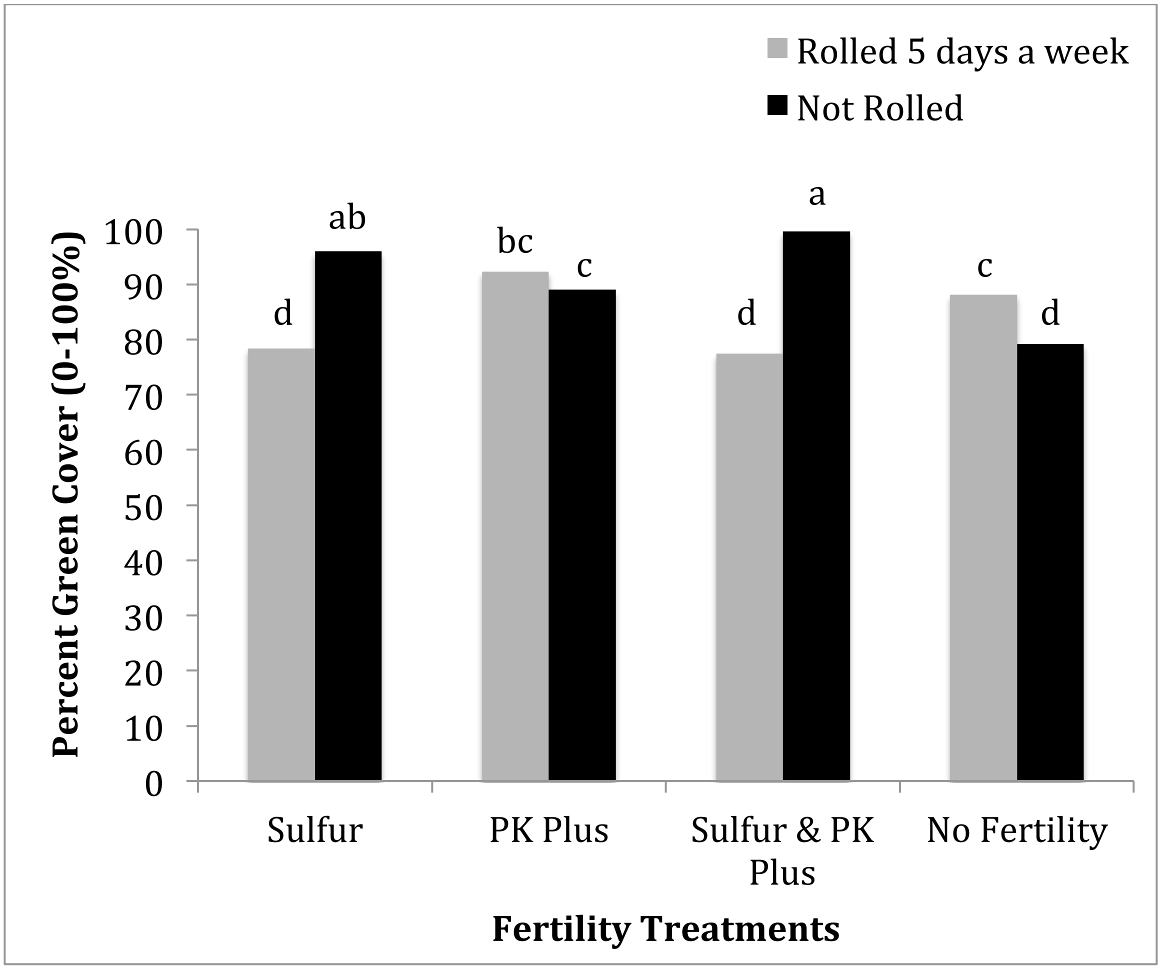

Figure 1.15: Effects of rolling and fertility treatments on percent green cover on an annual bluegrass putting green in Corvallis, OR on February 16, 2015. Sulfur DF at 12.22 Kg S ha^{-1}, PK Plus at 3.66 Kg H_3PO_3 ha^{-1}, and Sulfur DF+PK Plus was applied every other week. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at $P \leq 0.05$. Means represent 8 data points over 4 replications and 2 mineral oil treatments.

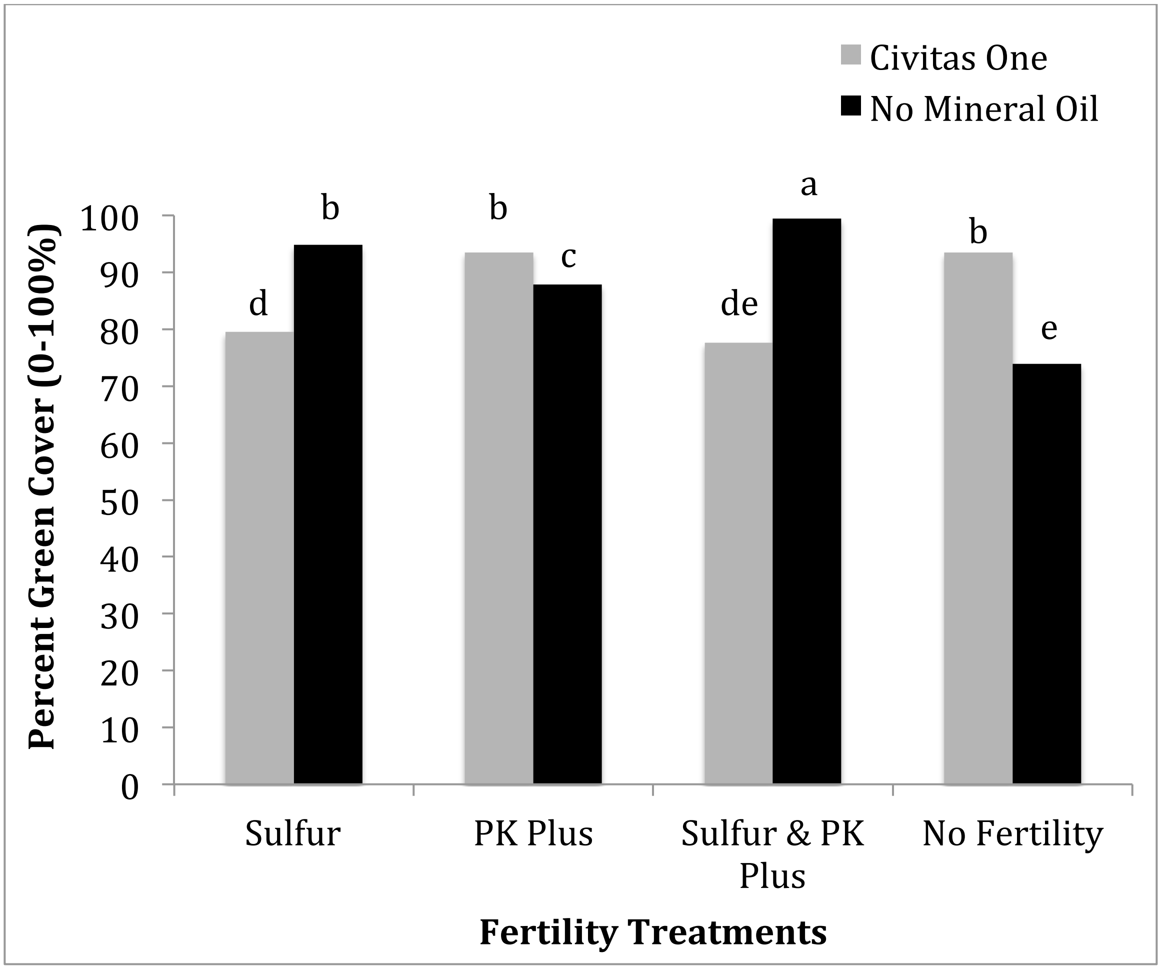

Figure 1.16: Effects of mineral oil and fertility treatments on percent green cover in Corvallis, OR on an annual bluegrass putting green on February 16, 2015. Civitas One 19.94 Kg a.i. ha^{-1}, Sulfur DF 12.22 Kg S ha^{-1}, PK Plus 3.66 Kg H_3PO_3 ha^{-1} and Sulfur DF+PK Plus were applied every other week. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at $P \leq 0.05$. Means represent 8 data points, across 4 replications and 2 rolling treatments.

Tables

Table 1.1 Analysis of Variance of percent disease, AUDPC, turf quality and percent green cover on annual bluegrass affected by rolling, mineral oil and fertility treatments in Corvallis, OR over two years.

Year 1		Percent Disease (0-100%)	AUDPC	Turf quality (1-9)	Percent green cover (0-100%)
Source of variation	DF	------------------------------ Pr > F ------------------------------			
Rolling (R)	1	ns	ns	*	ns
Oil (O)	1	***	***	***	***
Fertility (F)	3	***	***	***	***
R X O	1	*	ns	***	*
R X F	3	*	ns	ns	ns
O X F	3	***	***	*	***
O X R X F	3	ns	ns	ns	ns

Year 2		Percent Disease (0-100%)	AUDPC	Turf quality (1-9)	Percent green cover (0-100%)
Source of variation	DF	------------------------------ Pr > F ------------------------------			
Rolling (R)	1	**	**	**	*
Oil (O)	1	***	***	ns	*
Fertility (F)	3	***	***	***	**
R X O	1	***	***	***	***
R X F	3	***	***	ns	***
O X F	3	***	***	***	***
O X R X F	3	***	***	ns	ns

*** Significant at a 0.001 level of probability
** Significant at a 0.01 level of probability
* Significant at a 0.05 level of probability
ns Not significant at a 0.05 level of probability

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Chapter 3

Rolling and Biological Control Products Reduce Microdochium Patch on an Annual Bluegrass Putting Green

Abstract

Microdochium patch is a turfgrass disease in cool, humid regions caused by the pathogen *Microdochium nivale* (Fr.) Samuels & Hallett. Currently, fungicide applications are the predominant method of control. Increasing pesticide restrictions have generated concern regarding management of Microdochium patch. The objective of this research was to evaluate the effects of rolling in combination with biological control products in the absence of traditional fungicides on Microdochium patch incidence on an annual bluegrass (*Poa annua* L.) putting green. A two-year field trial was initiated in September 2013 and concluded in June 2015 on a sand-based putting green at the Oregon State University Lewis Brown Horticulture Farm, Corvallis, OR. Experimental design was a 2 by 2 by 4 randomized complete split-plot design with four replications. Factors included year, rolling and biological control treatments. Rolling treatments were applied five days a week and biological control treatments were applied every two weeks from September 26, 2013 to June 13, 2014 and again from September 22, 2014 to June 12, 2015. No traditional fungicides treatments were applied during the study. Biological control products included; Rhapsody (15.9 L ha^{-1}), ProVide (19.1 L ha^{-1}) + ReVive (1.3 Kg ha^{-1}) and BW136N applied initially at 24.4 Kg ha^{-1} and all subsequent applications at 18.3 Kg ha^{-1}

every other week compared to a control. The active ingredient in Rhapsody is *Bacillus subtilis* strain WST 713 and the active ingredients in BW136N are *Trichoderma harzianum* Rifai strain T-22 + *Trichoderma virens* strain G-41. ProVide and ReVive are a liquid soil amendment and humic acid respectively. Turf quality (1-9 scale, with 6 or greater considered acceptable) and percent disease (0-100%) data were collected weekly. In year one rolling, BW136N and Rhapsody were shown to reduce Microdochium patch disease. In year two rolling suppressed Microdochium patch incidence compared to plots without rolling. BW136N, followed by Rhapsody suppressed Microdochium patch disease compared to the remaining biological control products and the control. Area under disease progress curve data revealed that rolling was able to reduce disease compared to plots without rolling. BW136N and Rhapsody reduced AUDPC values in year one. In year two BW136N reduced AUDPC values, no differences were observed between the remaining products and the control treatment.

Introduction

Biological control can be defined as the complete or partial demise of plant pathogen populations through the use of other living organisms (Agrios, 2005). In turfgrass systems, the primary forms of biological control are the addition of microbes that suppress disease or organic matter to stimulate the growth of favorable microbes (Nelson, 2003). Microbial additions have been shown to provide control of turf pathogens such as the addition of *Pseudomonads* or their metabolites to control dollar spot (Powell et al., 2000, Horvath and Vargas, 2000) and

Microdochium patch (Dwyer and Vargas, 1999) on creeping bentgrass and also on annual bluegrass (Stahnke et al., 1997). *Trichoderma*, *Bacillus*, and *Pseudomonas* have also been shown to provide some control of Microdochium patch on a mixed stand of perennial ryegrass (*Lolium perenne*) and Kentucky bluegrass (*Poa pratensis*) (Raikes, 1997). While *Pseudomonas aureofaciens* is no longer available for turfgrass disease control, the *Bacillus* and *Trichoderma* genera are currently marketed for turfgrass use. No sources are readily available discussing the use of either *Bacillus* or *Trichoderma* to control Microdochium patch on annual bluegrass.

The bacterial genus *Bacillus* is a gram-positive bacterium used as a biological control agent for plant disease management of both soil and air-borne pathogens (Willey et al., 2014; Agrios, 2005). The mode of action has not been elucidated, although the genus *Bacillus* has been associated with plant growth promotion (Kumar et al., 2011), the synthesis of antibiotic compounds (Stein, 2005; Joshi and McSpadden Gardener, 2006; Yang et al, 2015) and the ability to induce systemic resistance in plants (Kloepper et al., 2004; Kumar et al., 2011). Recent genome sequence data of *Bacillus subtilis* reveals expression for antibiotic production, carbohydrate genes associated with fungal cell wall degradation and extracellular polymeric substance (biofilm) production (Wipat and Harwood, 1999; Guo et al., 2015). The species *B. subtilis* has been shown to suppress *M. nivale* in vitro and in vivo on a mixed stand of perennial ryegrass and Kentucky bluegrass (Raikes, 1997) and also Rhizoctonia large patch (*Rhizoctonia solani*) on mascarene grass (*Zoysia tenuifola*) when used to inoculate a compost (Nakasaki et al., 1998). No research is currently available concerning the effects of *Bacillus subtilis* in controlling Microdochium patch on annual bluegrass.

The fungal genus *Trichoderma* is known to possess plant pathogen suppression characteristics (Papavizas, 1985). *Trichoderma* is known to act as a parasite on other fungi by coiling around host hyphae and penetrating the chitin-containing fungal cell wells through the use of chitinolytic enzymes (Lorito et al., 1993; Webster and Weber, 2007). *Trichoderma* has also shown the ability to suppress both bacteria and fungi in vitro through the synthesis of antibiotic compounds notably; gliotoxin, viridian, trichodermin and peptide antibiotics (Dennis and Webster, 1971a and 1971b; Papavizas, 1985). Enzymes produced by *Trichoderma* used in combination with fungicides have shown a synergistic effect in vitro for the control of *Botrytis cinerea* fungal spore germination (Lorito et al., 1994).

Regarding turfgrass diseases, the species *T. harzianum* has been shown to suppress brown patch (*Rhizoctonia solani*) and pythium (*Pythium graminicola*) on creeping bentgrass (*Agrostis stolonifera*) putting greens (Lo et al., 1996) and control dollar spot (*Sclerotinia homeocarpa*) to levels equivalent to propiconazole when applied as a foliar spray on a weekly basis (Lo et al., 1997). Microdochium patch control on a mixed stand of perennial ryegrass and Kentucky bluegrass was observed in vitro and in the field with *T. harzianum* Rifai in a study in the United Kingdom (Raikes, 1997). To date, no data is available regarding the effects of *T. harzianum* on the control of Microdochium patch on annual bluegrass.

Compost additions have also shown promise in turfgrass disease suppression. Composts derived from a variety of sources have shown suppression of pythium (Craft and Nelson, 1996) on creeping bentgrass and dollar spot (Nelson and Craft, 1992) on a mixed stand of creeping bentgrass and annual bluegrass. Compost topdressing has been shown to suppress pink snow mold (*M. nivale*) to levels equivalent to fungicides on creeping bentgrass (Boulter et al. 2002) and compost teas have been reported to reduce Microdochium patch incidence from 0.6% to 0.04% on a golf course in San Francisco although the turfgrass species was not mentioned (Grobe, 2003). While the mode of action of these compost applications has not yet been elucidated, disease inhibition is often explained by an increase in beneficial microbes leading to an increase in competition, antibiotic metabolite production or induced systemic resistance (Scheuerell and Mahaffee, 2002; Nelson, 2003). To date, no reports of Microdochium patch suppression has been scientifically documented by compost additions on annual bluegrass.

Because golf course putting greens are often constructed using sand (USGA, 1993), the use of solid compost materials may prove to be problematic as they biodegrade leaving fine materials that could be detrimental to the drainage characteristics of a sand-based putting green. Therefore, aqueous compost extracts, commonly referred to as compost teas, are becoming more common in this particular application (Grobe, 2003). Specific problems associated with testing the effects obtained from compost teas are that they are often inconsistent in production and disease suppression potential (Horvath and Vargas, 2000; Scheuerell and Mahafee, 2002). A new liquid soil amendment (ProVide) derived from a controlled composting procedure is currently available with the objective to provide a consistent product with benefits similar to compost teas (Earthfort, 2015).

More recently, the cultural practice of rolling has been shown to influence disease incidence on turfgrass. Inguagiato (2009) reported a 5 to 6% decrease in anthracnose (*Colletotrichum cereale*) under moderate disease pressure on an annual bluegrass putting green when rolling every other day and Giordano (2012) observed that rolling a mixed stand of annual bluegrass and creeping bentgrass 5 days a week reduced AUDPC values for dollar spot incidence with an even greater decrease in AUDPC values when rolling occurred 2 times a day. In Corvallis, preliminary trials showed rolling was able to reduce Microdochium patch by as much as 75% on an annual bluegrass putting green (Mattox et al., 2014).

Researchers suspect a correlation to this cultural practice and changes in the soil microbial community of a sand-based putting green. Giordano (2013) revealed an increase in bacterial populations and a decrease in fungal populations within rolled plots using phospholipid fatty acid analysis. This decrease in fungal populations may explain the decrease in dollar spot activity on creeping bentgrass greens observed by Giordano (2013).

The objective of this study was to quantify the influence of the cultural practice of rolling on biological control products capacity to suppress Microdochium patch on an annual bluegrass putting green.

Methods and Materials

Experimental Area Construction

A field study was conducted at the Oregon State University Lewis-Brown Horticulture Farm in Corvallis, OR from September 26, 2013 to June 13, 2014 and again from September 22, 2014 to June 12, 2015. The putting green used for this research was constructed in April 2013 by placing 15 cm of USGA sand (USGA, 1993) directly on the native McBee silty clay loam soil (UC Davis, 2015) that was graded to a 2% surface slope. Annual bluegrass was established by collecting 13mm diameter by 76mm depth aerification cores from a predominantly annual bluegrass putting green at the Corvallis Country Club in Corvallis, OR. Cores were spread using a Meter-Matic topdresser (Turfco, NE Blaine, MN). Individual plots were 1.5m^2 and the total experiment area was 48m^2.

Experimental Design

Experimental design was a 2 by 2 by 4 randomized complete split-plot design with four replications. Individual plots were 1.5 m^2 plots and the total experiment area was 48 m^2. Factors included year (one and two), rolling (whole-plot) and biological control treatment (split-plot). Rolling was applied five days a week using a 122cm golf course putting green roller (Tru-turf, Queensland, Australia) and compared to a control. Biological control products; Rhapsody, Provide + ReVive and BW136N were applied every two weeks and compared to a control. Rhapsody (Bayer Crop Science, Monheim, Germany) was applied at 15.9 L ha^{-1} (0.154 Kg *Bacillus subtilis* strain WST 713 ha^{-1}). BW136N was applied initially at 24.4 Kg ha^{-1}

(0.28 Kg *Trichoderma harzianum* Rifai strain T-22 ha^{-1} and 0.15 Kg *Trichoderma virens* strain G-41 ha^{-1}) and all subsequent applications were applied at 18.3 Kg ha^{-1} (0.21 Kg a.i. ha^{-1} and 0.11 Kg a.i. ha^{-1}) (Bioworks, Victor, NY). ProVide, compost derived liquid soil amendment, was applied at 19.1 L ha^{-1} in combination with ReVive, a Leonardite derived humic acid, at a rate of 1.3 Kg ha^{-1} (0.09 kg a.i. ha^{-1}) (Earthfort, Corvallis, OR).

Treatments were applied every two weeks from September 26, 2013 to June 13, 2014 and again from September 22, 2014 to June 12, 2015. All treatments were applied with a CO_2-powered backpack sprayer with a 4 nozzle hand-held boom equipped with XR11015 nozzles using a pressure of 2.8 bars at the boom. Carrier volume was 814 L ha^{-1} and displacement speed was calibrated with a metronome.

Turfgrass Maintenance

The putting green was mowed as needed at a mowing height of 3.8 mm and clippings were removed. Annual nitrogen rates from April 2013 to March 2014 were 283 Kg N ha^{-1} and from April 2014 to March 2015 were 222 Kg N ha^{-1}. During the trial period, nitrogen applications were in the form of Urea (46N-0P-0K) and did not exceed 9.7 Kg N ha^{-1} every two weeks. Irrigation was applied as needed throughout the year; peak rates during summer heat stress were 2.54 cm per week. Topdressing was applied every two weeks from July 5, 2013 to September 05, 2013 and again from July 03, 2014 to September 02, 2014. Hollow-tine aerification using 13mm diameter tines on 5.1cm by 5.1cm spacing at a depth of 76mm was performed on September 18, 2013, and then on June 19 and September 16, 2014. No fungicide treatments other than the biological control products used in the trial were applied at any time throughout the study.

Response Variables

Response variables included percent disease, area under disease progress curve (AUDPC) and turfgrass quality. Percent disease data were collected weekly throughout the trial. Percent disease was a visual rating ranging from 0% to 100% collected from September 27th 2013 to June 13th 2014 and from October 2nd 2014 to June 12th 2015. The peak of disease was determined for both years and then percent disease observed on these dates only (March 13, 2014 and February 18, 2015) was presented (Figure 2.1).

To quantify disease development over the course of the experiments, area under disease progress curve (AUDPC) data was determined by taking the sum (Σ) of the average disease severity $[(y^i + y^{i+1})/2]$ between two observations (y^i) multiplied by the time interval between the observations $[t^{i+1}-t^i]$ using the formula $\Sigma[(y^i + y^{i+1})/2][t^{i+1}-t^i]$ as determined by Shaner and Finney (1977). AUDPC data for this trial was determined by using the percent disease data that was collected weekly from initial data collection (September 27, 2013 and October 02, 2014) up to the final data collection date (June 13, 2014 and June 12, 2015).

Turfgrass quality ratings were assigned using the NTEP rating system (Morris and Shearman, 2015) at the peak of disease (March 13, 2014 and February 18, 2015). Turfgrass quality ratings were determined by the amalgamation of five influences; color, density, uniformity, texture and damage due to stress or disease. Turfgrass quality ratings were 1-9 with a 1 rating given to dead turf, 9 given to ideal turf and a rating of 6 being considered acceptable for putting green turf (Morris and Shearman, 2015).

Statistical Analysis

Data were subjected to analysis of variance using SAS 9.2 Proc Mixed (SAS Institute Inc., Cary, N.C.). Factors within this analysis included year, rolling and biological control treatments. When main effects and interactions were significant Fisher's Protected Least Significant Difference (LSD) was used to separate individual means at a 0.05 level of probability. Initial analysis of variance observed a significant year main effect as well as interactions between year and the remaining factors for all of the response variables; therefore, data were analyzed and presented separately by year.

Results

Percent Disease

At the peak of disease in year one, an interaction was observed between the rolling by biological control treatments (Table 2.1). Rolling improved disease suppression among all biological control treatments (Figure 2.2). Rolling in combination with either Rhapsody or BW136N provided the least amount of disease (≤15%), followed by BW136N without rolling (29%), rolling without biological control products (30%), and ProVide+ReVive with rolling (31%). Rhapsody without rolling resulted in 43% disease, and finally the control (no biological control products or rolling) and ProVide+ReVive without rolling resulting in the highest percent disease (> 65%).

In year two, significant main effects for rolling and biological control on percent disease were detected (Tables 2.1). Rolling suppressed disease by 65% in comparison to the plots without rolling. Regarding the biological control treatments, BW136N provided the least amount of disease (28%), followed by Rhapsody with 41%, while ProVide+ReVive and the control had the highest disease activity (>55%).

Area Under Disease Progress Curve

Significant main effects of rolling and biological control products on AUDPC data were observed in year one (Tables 2.1). Rolling treatments provided lower AUDPC values compared to treatments without rolling (Table 2.1). The biological control treatments BW136N and Rhapsody AUDPC values provided significantly lower results compared to the Untreated and ProVide+ReVive treatments.

In year two, analysis of the AUDPC data identified significant main effects of rolling and biological control treatments (Tables 2.1). Similar to year one results, rolling treatments provided lower AUDPC values compared to treatments without rolling (Table 2.1). The biological control treatment BW136N provided a significantly lower AUDPC value compared to the other biological control treatments in the study. No differences between Rhapsody, Provide+ReVive and the untreated were observed in year two.

Turfgrass Quality

In year one significant differences were observed for the main effects of rolling and biological control treatments, no interactions were detected (Tables 2.1). Plots receiving the rolling treatments provided significantly higher turfgrass quality ratings (4.1) compared to plots without rolling (3.3) (Table 2.1). The BW136N treatment resulted in the highest overall turfgrass quality rating (4.3), followed by the Rhapsody treatment (3.9). ProVide+ReVive and the untreated had the lowest turfgrass quality ratings of 3.3. Neither rolling nor the biological control products provided acceptable quality ratings (≥6) according to the NTEP scale (Morris and Shearman, 2015).

In year two, significant turfgrass quality differences were observed for the main effects of rolling and biological control treatments. No interactions were detected (Tables 2.1). Similar to year one results, plots receiving rolling provided higher turfgrass quality ratings (3.3) compared to plots without rolling (2.3). The BW136N treatment resulted in the highest overall turfgrass quality rating (3.4). No differences were observed between Rhapsody, ProVide + ReVive and the control which produced quality ratings of 2.9, 2.4, and 2.4, respectively.

Discussion

Area under disease progress curves in both years suggests that rolling is able to suppress Microdochium patch disease compared to plots without rolling. This result supports earlier studies on the benefits of rolling in regards to disease control. Inguagiato (2009) reported a 5 to 6% decrease in anthracnose under moderate disease pressure on an annual bluegrass putting green when rolling occurred every other day and Giordano (2012) observed that rolling a mixed stand of annual bluegrass and creeping bentgrass 5 days a week reduced AUDPC values for dollar spot incidence with an even greater decrease in AUDPC values when rolling occurred 2 times a day.

In Corvallis, preliminary trials showed rolling was able to reduce Microdochium patch by as much as 75% on an annual bluegrass putting green (Mattox et al., 2014). This is in contrast to observations by Nikolai (2001) on creeping bentgrass where Microdochium patch incidence was greater on plots receiving rolling treatments. The differences in results between the study by Nikolai and this study might come from the fact that Microdochium patch was observed in the third week of the previous study and the benefits of rolling had not yet taken place. Giordano (2013) speculated that the effect of rolling on microbial populations in the soil might be the cause of disease control and it is likely that on sand greens the impact of rolling on microbial characteristics would take longer than three weeks to occur.

The BW136N and the Rhapsody treatments provided better disease control than ProVide+ReVive in the first year (>55% and >40% compared to 0% control respectively) and BW136N provided the greatest disease control (53%) followed by Rhapsody (31%) during the second year of the study. These results provide a greater level of control of Microdochium patch than was previously reported by Raikes (1997) where mycelial disks of the *Trichoderma* genus were able to suppress Microdochium patch between 6 and 7% and *B. subtilis* applied at $1 \times 10^9 - 1 \times 10^{10}$

bacterial cells per square meter was able to suppress Microdochium patch by 3% on a mixed stand of perennial ryegrass and Kentucky bluegrass sod. Raikes may have had less of an effect by the single treatment application compared to this study where the biological control products were applied bi-weekly. While the control provided by rolling and the biological control products BW136N and Rhapsody is encouraging, they are not yet able to achieve the 100% control levels demanded by golfers (Brame, 2000) that is currently achieved by traditional fungicides (Golembiewski and McDonald, 2011b).

Turfgrass quality was improved in both years where rolling was applied although not to levels considered acceptable on the NTEP scale. Nikolai (2001) did not observe any differences in turfgrass quality as a result of rolling 3 times a week on three different topdressed creeping bentgrass putting green constructions (85:15 sand:peat v/v; 80:10:10 sand:soil:peat v/v and sandy clay loam unamendend substrate consisting of 58% sand, 20.5% silt, and 21.5% clay by weight). This is in comparison to the negative effects on turfgrass quality observed by DiPaola and Hartwiger (1994) when rolling creeping bentgrass 4 or more times per week on both an USGA specified and a native soil putting green resulted in turfgrass wear compared to plots rolled 1 time per week after three weeks of rolling treatments (Hartwiger, 1996).

It is difficult to compare the results of this current study to previous studies as turfgrass quality in this study was more of a function of the influence of rolling on Microdochium patch incidence than on the influence of lightweight rolling on overall turfgrass quality. Nevertheless, it can be concluded that rolling five days a week was not a detriment to turfgrass health in this study and indeed improved turfgrass quality during both years of the study.

BW136N provided the highest turfgrass quality among the biological control treatments. Similar to the results produced by rolling, turfgrass quality was greatly affected by percent disease incidence due to the high level of Microdochium patch occurrence. Even though BW136N was able to reduce Microdochium patch incidence in this study, there was still > 20% disease during both years with this treatment, resulting in turfgrass quality ratings only achieving 4.3 and 3.4 at the peak of disease in both years of this study.

Conclusion

In year one, rolling in combination with either the biological control product BW136N or Rhapsody was shown to reduce Microdochium patch disease. In the second year, rolling suppressed Microdochium patch incidence compared to plots without rolling, and BW136N as well as Rhapsody suppressed Microdochium patch disease. Area under disease progress curve data revealed that rolling reduced disease progression over time compared to plots without rolling. BW136N was also effective at reducing the AUDPC for Microdochium patch progression during both years of this study. Rhapsody applications reduced the AUDPC value in the first but not the second year.

Even though the treatments used in this study did not provide results equivalent to those often obtained from traditional fungicide products, it can be speculated that if future legal constraints result in chemical disease control no longer being an option, even the limited disease control observed in this study would be considered superior to no control at all. Future research may also find that the cultural practice of rolling or the use of biological controls such as BW136N or Rhapsody might be used in combination or in rotation with other disease management practices such as chemical controls in order to decrease the overall use of fungicides.

Figures

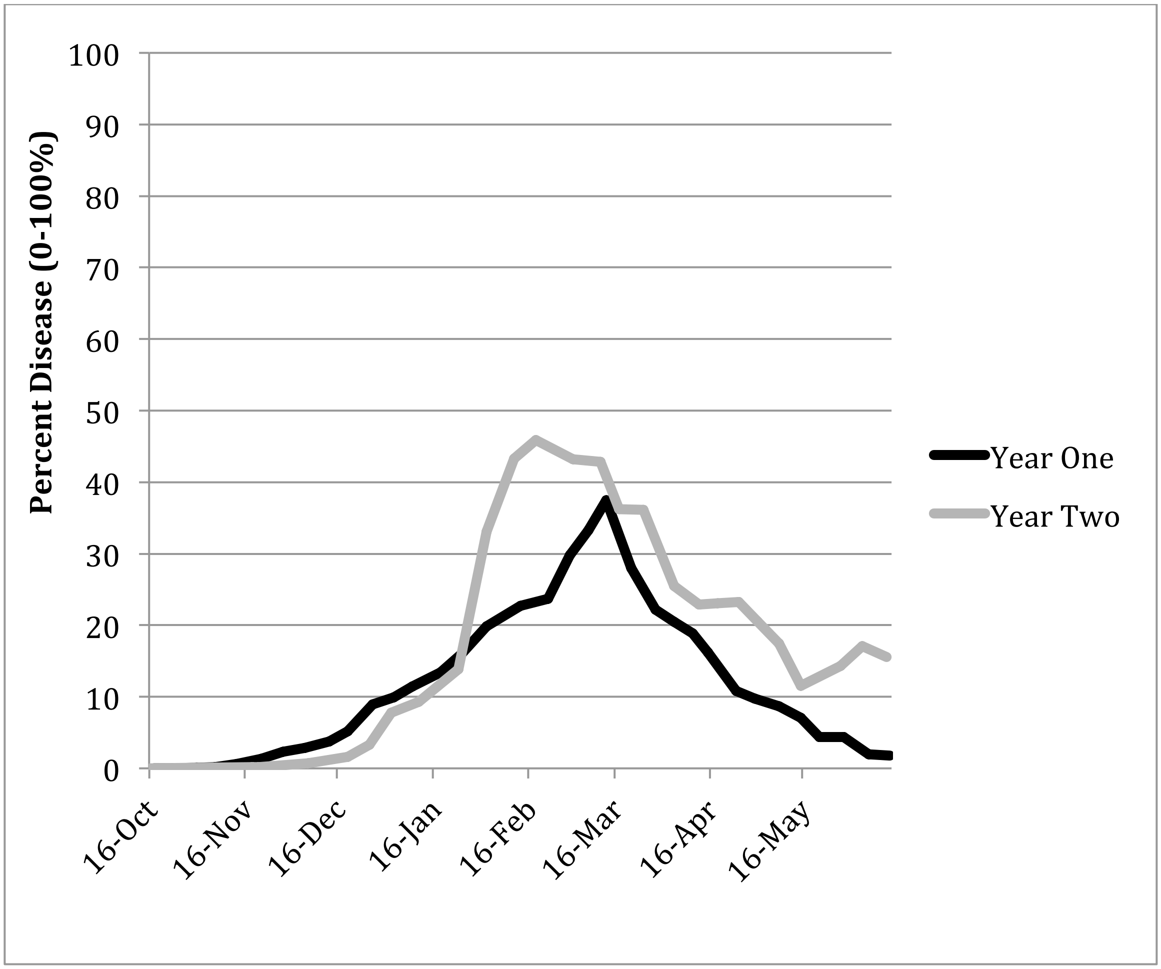

Figure 2.1: Microdochium patch development over time on an annual bluegrass putting green in Corvallis, OR over two years. Disease development over time measured as total combined disease on all plots from the onset of disease to the conclusion of the experiments for each year of a two-year experiment in Corvallis, OR.

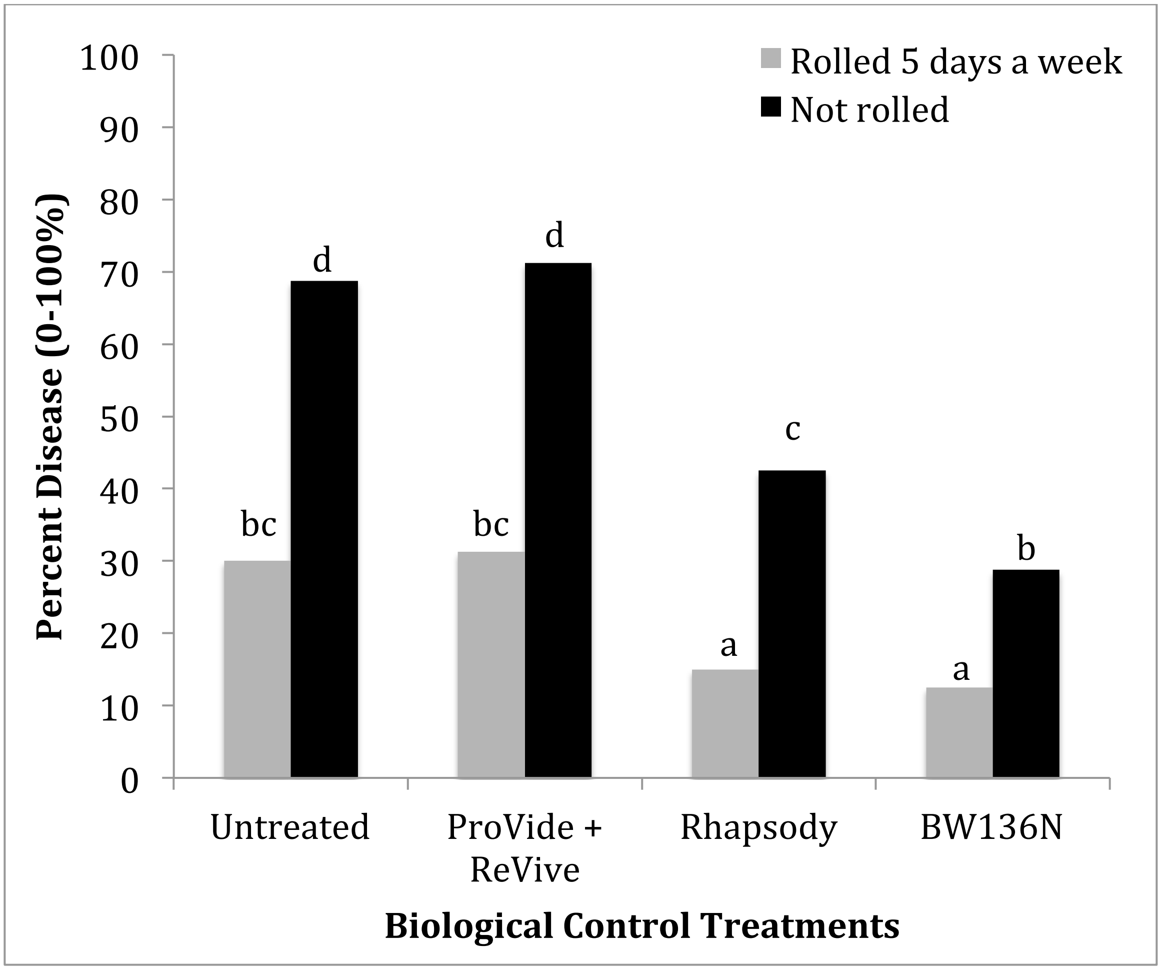

Figure 2.2: Effects of rolling and biological control products on percent disease on an annual bluegrass putting green in Corvallis, OR on March 13, 2014. ProVide (19.1 L/ha^{-1}) + ReVive (1.3 Kg ha^{-1}), Rhapsody (15.9 L ha^{-1}) and BW136N (initially 24.4 Kg ha^{-1} and subsequently 18.3 Kg ha^{-1}) was applied every two weeks. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at $P \leq 0.05$. Means represent 4 data points over 4 replications.

Tables

Table 2.1. Analysis of variance and effect of rolling and biological control products on percent disease, area under disease progress curve (AUDPC), and turf quality of on annual bluegrass putting green in Corvallis, OR from September 2013 to March 2015.

		-----------Year 1[z]------------			------------Year 2[y]------------		
		AUDPC	Percent Disease	Turf Quality	AUDPC	Percent Disease	Turf Quality
Source of Variation	DF	--------------------------------------- *Pr* > F			--------------------------------		
Rolled (R)	1	*	**	**	**	***	***
Biocontrol (B)	3	**	***	***	***	***	**
R by B	3	ns	*	ns	ns	ns	ns

	-----AUDPC------		Percent Disease		Turfgrass Quality	
Rolling	Year 1	Year 2	Year 1[x]	Year 2	Year 1	Year 2
5 times week	10.0 a[w]	27.3 a	22% a	24% a	4.1 a	3.3 a
Not rolled	23.4 b	56.6 b	53% b	68% b	3.3 b	2.3 b
Biocontrol						
BW136N[v]	9.6 a	22.8 a	21% a	28% a	4.3 a	3.4 a
Rhapsody[u]	11.5 a	42.4 b	29% a	41% b	3.9 b	2.9 b
ProVide + ReVive[t]	21.3 b	52.2 b	51% b	56% c	3.3 c	2.4 b
Untreated	24.5 b	50.4 b	49% b	59% c	3.3 c	2.4 b

*** Significant at a 0.001 level of probability

** Significant at a 0.01 level of probability

* Significant at a 0.05 level of probability

ns Not significant at a 0.05 level of probability

[z] Year one trial began on 26 September 2013 and concluded on 12 April 2014.

[y] Year two trial began on 22 September 2014 and concluded on 12 June 2015.

[x] An interaction between rolling and biological control treatments was observed for year one percent disease data. The means are included here for comparison only.

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

[v] BW136N applied initially at 24.4 Kg ha^{-1} and subsequently applied at 18.3 Kg ha^{-1} every two weeks.

[u] Rhapsody applied at 15.9 L ha^{-1} every two weeks.

[t] ProVide + ReVive applied at 19.1 L ha^{-1} and 1.3 Kg ha^{-1} respectively every two weeks.

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Chapter 4

The Influence of Nitrogen and Iron Sulfate on the Incidence of Microdochium Patch in the Absence of Fungicides on an Annual Bluegrass Putting Green

Abstract

Microdochium patch is a turfgrass disease in cool, humid regions caused by the pathogen *Microdochium nivale* (Fr.) Samuels & Hallett. Currently, fungicide applications are the predominant method of control. Increasing pesticide restrictions have generated concern regarding management of Microdochium patch. The objective of this research was to evaluate the effects of different rates of nitrogen and iron sulfate on Microdochium patch development on trafficked annual bluegrass (*Poa annua* L.) putting greens in the absence of fungicides. A field experiment was initiated in September 2013 and concluded in April 2015 on a sand-based putting green at the Oregon State University Lewis Brown Horticulture Farm, Corvallis, OR. Experimental design was a 2 by 3 by 5 randomized complete block design with four replications. Factors included year, nitrogen, and iron sulfate rates. Treatments were applied every two weeks from September 26, 2013 to April 12, 2014 and again from September 22, 2014 to April 12, 2015. No fungicides treatments were applied during the study. Nitrogen rates were 0.0, 4.88 and 9.76 Kg N ha^{-1} and rates of iron sulfate were 0.0, 12.21, 24.41, 48.82, and 97.65 Kg $FeSO_4$ ha^{-1}. Traffic simulation was performed by walking over the plots with golf shoes 5 days a week to replicate 76 rounds of golf per day. Turf quality and turf color (1-9 scale, with 6 or greater considered acceptable), and percent disease (0-100%) data were collected weekly. Treatments receiving 0.0 Kg N ha^{-1} in combination with 48.82 Kg

$FeSO_4$ ha^{-1}, or 0.0 and 4.88 Kg N ha^{-1} combined with 97.65 Kg $FeSO_4$ ha^{-1} provided the greatest disease control, less than 1% disease. In spite of the disease suppression observed, no treatment received a turfgrass quality rating of 6.0 or greater. Low turfgrass quality ratings were attributed either to turf thinning or blackening of the shoots resulting from iron sulfate applications. Regarding color quality, treatments not receiving nitrogen resulted in the lowest ratings.

Introduction

Turfgrass nutrition has traditionally been linked to Microdochium patch incidence. The compendium of turfgrass diseases states that high levels of nitrogen fertility increase susceptibility of turfgrass to Microdochium patch, referring to nitrogen's impact on leaf succulence although no guidelines are provided as to how much constitutes a high level (Smiley et al, 1992). Couch (1995) states that increasing levels of nitrogen might create conditions more conducive for disease by either increasing turf succulence or allowing for longer growth (that may lead to an increase in humidity) during the winter months. An early study looking at the effects of nitrogen on Microdochium patch incidence considered four different rates of ammonium sulfate additions (0, 17.8, 35.6 and 71.2 Kg N ha^{-1}) to annual bluegrass turf in July, August and September and measured the percent area affected by Microdochium patch the following spring. It was found that the following March rating date resulted in 0% disease for the plots receiving no nitrogen, 1% disease for the lowest annual dose (53.4 Kg N ha^{-1}) compared to 31% for the highest dose (214 Kg N ha^{-1}) (Anonymous, 1956). This was evidence that higher nitrogen rates resulted in more disease, but also that some nitrogen in the late summer did not necessarily correlate to dramatic levels of Microdochium patch the following spring. It is currently difficult to obtain published scientific work referring to how low rates of nitrogen (for instance 4.88 and 9.76 Kg N ha^{-1} 2 wks^{-1}) during the late fall and winter period affects Microdochium patch activity.

Soil pH is often reported to have an effect on Microdochium patch incidence with higher pH considered conducive to disease (Couch, 1995; Smiley et al., 1992; Smith, 1958). A study on colonial bentgrass (*Agrostis tenuis*) showed that annual sulfur rates of 224 Kg ha^{-1} resulted in complete control of Microdochium patch (Brauen et al., 1975). Although no direct causation was offered, it can be speculated that the sulfur caused a decrease in pH due to sulfur oxidation where the bacteria *Thiobacillus* converted sulfur to sulfuric acid. The newly formed sulfuric acid would have the potential to dissociate in the soil resulting in a release of hydrogen ions, ultimately lowering the pH (McCarty et al., 2003). Unlike sulfur that requires microorganisms such as *Thiobacillus* for oxidation to occur (and is therefore temperature dependent), iron sulfate reacts readily with water to release hydrogen ions resulting in a reduction of soil pH (Carrow et al., 2001). The use of iron sulfate to manage turfgrass diseases notably Microdochium patch is a common practice in Europe (Baldwin, 1989; Mabbett, 2014; and Pitchcare, 2015). Even though there is a great deal of anecdotal evidence available, little published scientific research exists that quantifies the effects of iron sulfate applications regarding Microdochium patch disease. A one year study in the UK on a mixed stand of turfgrass (*A. tenuis, P. annua* and *Festuca rubra commutata*), showed that iron sulfate applied at a 10 Kg ha^{-1} rate every five weeks from August to February for a total of 60 Kg ha^{-1} applied was able to decrease Microdochium patch (26% disease cover) compared to the control (45% disease cover) (Oostendorp, 2012). No decrease in pH was observed in this 20-week study. The author concluded that perhaps the pH was affected only near the soil surface and therefore did not affect soil acidity when tested using a composite soil sample.

Other than the effect on pH by iron sulfate, it has been proposed that disease suppression might be due to iron sulfate "hardening" the turfgrass (Cole, 1930; Watson, 2008 and Oostendorp, 2012). Turfgrass hardening is a term that is used to describe physiological events that take place in the turfgrass plants that allow them to lower the critical temperature for injury due to cold or freezing temperatures (Beard and Beard, 2005) and has also been used to describe the ability of turfgrass to be conditioned to better resist disease (Watson, 2008). In regards to Microdochium patch, iron sulfate may be hardening turfgrass by causing desiccation of the leaf due to osmotic effects (Cole, 1930). This desiccation effect is similar to the hypothesis by Brauen et al. (1975) that thin turf would be less susceptible to Microdochium patch incidence than succulent turf (Couch, 1995).

In a recent study on the effects of iron sulfate, sulfur and 10% chelated iron (Fe-EDTA) on dollar spot incidence on "Penn A4" creeping bentgrass putting greens, it was observed that only the chelated iron and iron sulfate treatments were able to reduce dollar spot. This led to the author concluding that iron was most likely responsible for the reduction in dollar spot (Reams, 2013). An earlier study looking at iron applications on wheat surmised that iron might have been providing a fungistatic role regarding stem rust (*Puccina graminis* var. *tritici*) (Forsyth, 1957). Incidentally, in addition to looking at the role of iron sulfate and dollar spot disease, Reams (2013) also observed that high rates of iron sulfate (48.8 Kg ha^{-1} 2wks^{-1}) reduced annual bluegrass populations.

One of the key studies to take place regarding turfgrass nutrition and Microdochium patch incidence was performed by Brauen et al. (1975) on Colonial bentgrass putting greens. Four levels of Nitrogen rates were used in their study (0, 293, 586, and 976 Kg ha^{-1}) in combination with four sulfur levels (0, 56, 112, and 224 Kg ha^{-1}). The results of this study indicated that all treatments containing sulfur were able to suppress Microdochium patch and the highest sulfur rate (224 Kg ha^{-1}) resulted in complete control of Microdochium patch (Brauen et al., 1975). Turfgrass stress due to golfer foot traffic was not included in this study; therefore it is not possible to conclude that the effective Microdochium patch suppressive combination of nitrogen and sulfur would be able to provide nutrition that would also be able to provide the nutrients needed by turfgrass to recuperate from the actual stresses observed on the golf course. It is also important to note that the lowest rate of nitrogen used in Brauen's study (293 Kg N ha^{-1}) would be considered high for most annual bluegrass putting greens today.

The objective of this study was to quantify the effects of iron sulfate and urea application rates on the incidence of Microdochium patch on an annual bluegrass (*P. annua L.)* putting green under replicated golfer traffic in the absence of fungicide applications.

Materials and Methods

Experimental Area Construction

A field study was conducted at the Oregon State University Lewis-Brown Horticulture Farm in Corvallis, OR from September 26, 2013 to April 12, 2014 and again from September 22, 2014 to April 12, 2015. The putting green used for this research was constructed in April 2013 by placing 15 cm of USGA specification (USGA, 1993) sand directly on the native McBee silty clay loam (UC Davis, 2015), which was graded to a 2% slope. Annual bluegrass was established by collecting 13mm diameter by 76mm depth aerification cores from a predominantly annual bluegrass putting green in Corvallis, OR. Cores were spread using a Meter-Matic topdresser (Turfco, NE Blaine, MN).

Experimental Design

Experimental design was a 2 by 3 by 5 randomized complete block design with four replications. Individual plots were 1.5 m^2 plots and the total experiment area was 90 m^2. Factors included year (one and two), urea (46N-0P-0K) rate (0.0, 4.88 and 9.76 Kg N ha^{-1}) and iron sulfate heptahydrate ($FeSO_4$ $7H_20$, 20% Fe, 12% S) rate (0.0, 12.21, 24.41, 48.82, 97.65 Kg product ha^{-1}). Treatments were applied every two weeks from September 26, 2013 to April 12, 2014 and again from September 22, 2014 to April 12, 2015. All treatments were applied with a CO_2-powered backpack sprayer with a 4 nozzle hand-held boom equipped with XR11015 nozzles using a pressure of 2.8 bars at the boom. Carrier volume was 814 L ha-1 and displacement speed was calibrated with a metronome. In order to replicate golf conditions, simulated golfer traffic representing 76 rounds of golf per day was performed by walking over the plots using golf shoes following the protocol set out by Hathaway et al. (2005).

Turfgrass Maintenance

The green was mowed as needed at a mowing height of 3.8 mm and clippings were removed. Preceding the first year of the trial, 234 Kg N ha^{-1} was applied from April to August using a compound fertilizer (28N-2.2P-15K) (Andersons 28-5-18, Maumee, OH) in order to establish the green. Preceding the second year of the trial, 80 Kg N ha^{-1} applied using a compound fertilizer (28N-2.2P-15K) (Andersons 28-5-18, Maumee, OH) was used to recover the green from the damage of the first year.

Topdressing was applied every two weeks from July 5, 2013 to September 05, 2013 and again from July 3, 2014 to September 02, 2014. Hollow-tine aerification using 13mm diameter tines on 5.1cm by 5.1cm spacing at a 76mm depth was performed on September 18, 2013, and again on June 19 and September 16, 2014. No fungicides were applied at any time throughout the study. Irrigation was applied as needed throughout the year; peak rates during summer heat stress were 2.54 cm per week.

Response Variables

Response variables included percent disease, area under disease progress curve (AUDPC), turfgrass color and quality, as well as soil pH, iron (Fe) and sulfur (S) levels. Percent disease data were collected weekly throughout the trial. Percent disease was a visual rating ranging from 0% to 100% collected from September 27, 2013 to April 14, 2014 and October 2, 2014 to April 12, 2015. In order to compare the effects of the treatments during the period when the disease incidence was at its highest level, the peak of disease was determined for both years (Figure 3.1) and then percent disease observed on these dates only (February 13th, 2014 and February 11th, 2015) was presented. To quantify disease development over the course of the experiments, area under disease progress curve (AUDPC) data was determined by taking the sum (Σ) of the average disease severity $[(y^i + y^{i+1})/2]$ between two observations (y^i) multiplied by the time interval between the observations $[t^{i+1}-t^i]$ using the formula $\Sigma[(y^i + y^{i+1})/2][t^{i+1}-t^i]$ as determined by Shaner and Finney (1977). AUDPC data for this trial was determined by using the percent disease data that was collected weekly from initial data collection (September 27, 2013 and October 02, 2014) up to the date of peak disease (February 13, 2014 and February 11, 2015).

Turfgrass color and quality ratings were assigned using the NTEP rating system (Morris and Shearman, 2015) at the peak of disease in February of 2014 and 2015. For color this is a 1-9 scale with a 1 rating given to turf straw brown in color and a 9 given to dark green turf. Turfgrass quality ratings were determined by the amalgamation of five influences; color, density, uniformity, texture and damage due to stress or disease. Similar to color, turfgrass quality rating were 1-9 with a 1 rating given to dead turf, 9 given to ideal turf and a rating of 6 being considered acceptable for putting green turf (Morris and Shearman, 2015).

Soil samples were collected on May 17, 2014 and May 18, 2015. Twenty-five samples from each plot were collected to a depth of 76mm using a soil core sampler 16mm in diameter. The top 12mm was removed in order to separate the thatch from the soil collected. The samples were mixed, placed in plastic bags and sent to a soil-testing lab (A and L labs, Modesto, CA) for analysis of soil acidity, sulfate levels and available iron.

Statistical Analysis

Data were subjected to analysis of variance using SAS 9.2 Proc Mixed (SAS Institute Inc., Cary, N.C.). Factors within this analysis included year, urea rate and iron sulfate rate. When main effects and interactions were significant Fisher's Protected Least Significant Difference (LSD) was used to separate individual means at a 0.05 level of probability. Initial analysis of variance observed significant year or interactions between year and the remaining factors for all of the response variables; therefore, data were analyzed and presented separately by year.

Results

Percent Disease

At the peak of disease for the first year of the study, main effect differences were observed for both the nitrogen and iron sulfate (Table 3.1). The highest rates of iron sulfate (97.65 Kg FeSO4 ha^{-1} 2wks^{-1}) resulted in the lowest disease incidence with 1.3%, followed by the 48.82 Kg FeSO4 ha^{-1} 2wks^{-1} rate with 22.5% disease, followed by the 24.41 Kg FeSO4 ha^{-1} 2wks^{-1} rate with 37.9% Microdochium patch, followed by the 12.21 Kg FeSO4 ha^{-1} $2wks^{-1}$ rate with 48.8% disease and finally 57.5% disease in treatments not receiving iron sulfate (Table 3.2). Concerning nitrogen, treatments not receiving nitrogen and the low rate of nitrogen (4.88 Kg N ha^{-1}) were not significantly different from each other and resulted in less than 30% disease incidence compared to treatments receiving the highest rate of nitrogen (9.76 Kg N ha^{-1} $2wks^{-1}$) resulting in the highest level disease (> 40%) (Table 3.2).

At the peak of disease for year two, a significant interaction between the nitrogen and iron sulfate variables was observed (Table 3.1). The lowest disease incidence was observed on plots receiving 97.65 Kg FeSO4 regardless of nitrogen treatments as well as in plots receiving 48.82 Kg $FeSO_4$ in combination with 0.0 or 4.88 Kg N ha^{-1} (Figure 3.2). Treatments receiving 0.0 Kg N ha^{-1} in combination with 48.82 or 97.65 Kg $FeSO_4$ ha^{-1} as well as the combination of 4.88 Kg N ha^{-1} and 97.65 Kg $FeSO_4$ ha^{-1} resulted in the lowest disease populations, less than 0.5% Microdochium patch. Treatments not receiving iron sulfate resulted in the highest disease regardless of nitrogen rate (≥ 50%). Generally speaking, nitrogen applied at 9.76 Kg N ha^{-1} in combination with 12.21, 24.41 or 48.82 Kg $FeSO_4$ ha^{-1} increased disease activity in comparison to the 0.0 and/or the 4.88 Kg N ha^{-1} rate.

Area Under Disease Progress Curve

For the first year of the study, differences between the nitrogen rates and iron sulfate rates were observed for AUDPC values (Table 3.1). Treatments receiving 0.0 and 4.88 Kg N ha^{-1} every 2 weeks produced the lowest AUDPC values, compared to the highest rate of nitrogen used in this study (9.76 Kg N ha^{-1}) (Table 3.2).

Concerning iron sulfate, with the exception of the lowest rate (12.21), the AUDPC value decreased as the rate of iron sulfate increased with the 97.65 Kg $FeSO_4$ ha^{-1} rate resulted in the lowest AUDPC value. The 12.21 rates and the control produced the largest AUDPC values.

For the second year of AUDPC results, trends were similar to the first year with differences between the nitrogen and iron sulfate rates being observed (Table 3.1). Again, treatments receiving 0.0 and 4.88 Kg N ha^{-1} every 2 weeks produced the lowest AUDPC values (Table 3.2). Concerning iron sulfate, the highest rate (97.65) produced the lowest AUDPC values, followed by the 48.82 rate, then the 24.41 and 12.21 rate and finally the control, which produced the greatest AUDPC values (Table 3.2).

Color Quality

Concerning turfgrass color quality at the peak of disease for year one, a significant interaction between the nitrogen and iron sulfate rates was observed (Table 3.1). In general, whenever nitrogen was included in the treatments, color ratings improved across the individual iron sulfate rates (Figure 3.3). Treatments that included both nitrogen, regardless of the rate, and iron sulfate provided the highest color ratings, all receiving ratings greater than acceptable (> 6.0) on the NTEP scale. Treatments receiving 4.88 or 9.76 Kg N ha^{-1} in the absence of iron sulfate additions, received acceptable color quality ratings of 6.3 and 7.0 respectively. The lowest color quality rating observed was assigned to the combination of 0.0 Kg N ha^{-1} and 97.65 Kg $FeSO_4$ ha^{-1} resulting in a 3.1 color quality rating.

Concerning turfgrass color quality at the peak of disease for year two, a significant interaction between the nitrogen and iron sulfate rates was observed (Table 3.1). Similar to year one findings, whenever nitrogen was included in the treatments, turfgrass color improved across the various iron sulfate rates (Figure 3.4). Again, all treatment combinations that included nitrogen, regardless of rate, received acceptable ratings (≥ 7.0). Treatments receiving 4.88 or 9.76 Kg N ha-1 in the absence of iron sulfate additions, received acceptable color quality ratings of 7.4 and 7.9 respectively. Similar to year one, the lowest color rating observed was the combination of 0.0 Kg N ha^{-1} and 97.65 Kg $FeSO_4$ ha^{-1} receiving a 3.8 color quality rating.

Turfgrass Quality

Turfgrass quality results indicated a significant interaction between the nitrogen and iron sulfate rates at the peak of disease for year one (Table 3.1). The treatment combination of 4.88 Kg N ha^{-1} and 97.65 Kg $FeSO_4$ ha^{-1} resulted in the highest quality rating (4.5) followed by the combination of 9.76 Kg N ha-1 and 97.65 Kg FeSO4 ha-1 (4.1). All other treatment combinations resulted in turfgrass quality ratings < 4.0 with the lowest rating resulting in the combination of 0.0 Kg N ha-1 and 97.65 Kg FeSO4 ha-1 (2.4) (Figure 3.5).

Year two turfgrass quality analysis resulted in a significant interaction between the nitrogen and iron sulfate rates (Table 3.1). The group with the highest turfgrass quality ratings was observed for 4.88 Kg N ha^{-1} treatments combined with 24.41, 48.82 or 97.65 Kg FeSO4 ha^{-1} with ratings of 4.3, 4.8 and 4.6 respectively. This group was followed by treatment combinations of 4.88 Kg N ha-1 with 12.21 Kg FeSO4 ha^{-1} (4.1) and the combination of 9.76 Kg N ha-1 and 97.65 Kg N ha^{-1} (4.1). All other treatment combinations resulted in turfgrass quality ratings < 4.0 with the lowest rating resulting in the combination of 0.0 Kg N ha-1 and 97.65 Kg FeSO4 ha^{-1} (2.0) (Figure 3.6).

Soil Test Results

At the conclusion of the first year, a significant difference was observed concerning pH in regards to the nitrogen treatments (Table 3.1). The 0.0 Kg N ha^{-1} rate resulted in the lowest pH (5.75), followed by 4.88 Kg N ha^{-1} (5.82) and 9.76 Kg N ha^{-1} (5.85) (Table 3.2). Concerning Fe, a significant difference was observed in regards to the nitrogen treatments (Table 3.1), with no nitrogen additions resulting in the highest level of Fe observed in the soil tests compared to the 4.88 and 9.76 Kg N ha^{-1} rates (Table 3.2). Concerning sulfur (measured as sulfate), a significant difference was observed in regards to the iron sulfate treatments (Table 3.1). The 97.65 Kg FeSO4 ha^{-1} rate resulted in significantly higher sulfate soil test levels compared to all other treatments (Table 3.2).

At the conclusion of the second year, a significant difference was observed concerning pH in regards to the iron sulfate treatments (Table 3.1). The 97.65 Kg FeSO4 ha^{-1} rate resulted in the lowest pH (5.51) followed by the 48.82 Kg FeSO4 ha^{-1} rate (5.58) (Table 3.2). The remaining treatments (0.0, 12.21, and 48.82) were not significantly different with pH levels of 5.66, 5.64 and 5.66 respectively. Concerning Fe levels, no significant differences were observed in year two of the study (Table 3.1). Concerning sulfur, a significant difference was observed in regards to the iron sulfate treatments (Table 3.1). The 97.65 Kg FeSO4 ha^{-1} rate resulted in a significantly higher sulfate soil test level compared to all other treatments (Table 3.2). This was followed by the 48.82 and 24.41 Kg FeSO4 ha^{-1} rate, and then the 12.21 and 0.0 Kg $FeSO_4$ ha^{-1} rate. The 0.0 Kg FeSO4 ha^{-1} rate resulted in the lowest overall sulfate level.

Discussion

Area under disease progress curves and percent disease data analysis showed that a low rate of nitrogen (4.88 Kg N ha^{-1}) applied every two weeks during the winter period did not increase disease populations in comparison to the plots that did not receive nitrogen. The low nitrogen rate in fact frequently provided slight reductions in percent disease. Nearly every turfgrass disease book, publication, or resource refers to the risk of winter nitrogen fertilization applications associated with Microdochium patch disease incidence (Smiley et al., 1992; Couch 1995 and Vargas 2005). Early research showed that ammonium sulfate additions of 214 Kg N ha^{-1} to annual bluegrass turf in the summer resulted in 31% Microdochium patch observed the following spring compared to 0% in plots not receiving nitrogen (Anonymous, 1956). However, other research showed a decrease in Microdochium patch on colonial bentgrass greens as annual urea applications levels increased from 293, 586 and 976 Kg N ha^{-1} in the absence of sulfur additions when applied from February to December (Brauen et al., 1975). In this study, bi-weekly applications of 9.76 Kg N ha^{-1} resulted in an increase in Microdochium patch compared to the 4.88 Kg N ha^{-1} rate, but the 4.88 Kg N ha^{-1} rate did not increase Microdochium patch compared to no nitrogen additions. Perhaps in climates where annual bluegrass grows throughout the winter, turfgrass needs to have nitrogen to combat stresses such as disease and low bi-weekly rates of urea (4.88 Kg N ha^{-1}) benefit turf health without causing a detriment to Microdochium patch incidence.

Regarding iron sulfate applications, this study has shown that as rates of iron sulfate increase, Microdochium patch incidence decreases on an annual bluegrass putting green. A similar result was obtained by Oostendorp (2012) on a mixed stand of putting green turf (*A. tenuis, P. annua, and Festuca rubra commutata*) where 10 Kg FeSO4 ha^{-1} was applied every five weeks for 7 months resulting in a 58% reduction in Microdochium patch. On creeping bentgrass, a reduction in dollar spot (*Sclerotinia homeocarpa*) was observed with bi-weekly applications of iron sulfate at 48.8 Kg ha^{-1} over 6 months in the summer (Reams, 2013). The cause of the reduction in fungal disease is not clear, however possible explanations are that iron sulfate is decreasing the pH (Carrow et al., 2001) thus causing the environment to be less conducive to Microdochium patch invasion (Smith, 1958), iron toxicity might be responsible (Reams, 2013) or hardening of the leaves might be causing the leaves to become less succulent and less prone to disease (Cole, 1930; Watson, 2008; Danneberger, 2009).

All iron sulfate treatments (0.0, 12.21, 24.41, 48.82, and 97.65 Kg ha^{-1}) that were applied in combination with either the 4.88 or 9.76 Kg N ha^{-1} treatments resulted in higher color quality compared to the same iron sulfate treatments in the absence of nitrogen. In addition, when iron sulfate was included, color quality was not significantly improved compared to the same rate of nitrogen in the absence of iron sulfate. This observation is supported through experiments by Kussow (1995) where two summer applications of compound nitrogen and iron fertilizers with rates ranging from 4.88 to 67.34 Kg N ha^{-1} and 0 to 58.56 Kg Fe ha^{-1} were applied to creeping bentgrass resulting in nitrogen accounting for 94% of the variation in bentgrass color. An earlier study on winter applications of nitrogen and iron on creeping bentgrass in Virginia revealed that monthly applications of 1.2 Kg Fe ha^{-1} in Oct., Nov., Dec., and Feb. resulted in lower color quality ratings than the same treatments in combination with nitrogen applications of 50 Kg N ha^{-1} (Snyder, 1973). Iron treatments alone did result in higher color quality ratings than nitrogen treatments alone on the February rating date (Snyder, 1973) suggesting that iron may play a larger role in the winter months when creeping bentgrass is growing much slower than in the summer months. The differences observed between the previous study on creeping bentgrass and this study on annual bluegrass may be due to milder temperatures in Oregon and annual bluegrass's ability to grow at lower temperatures than bentgrass (Vargas and Turgeon, 2004).

It was also observed that the highest rates of iron sulfate (48.82 and 97.65 Kg $FeSO_4$ ha^{-1}) used in this study caused blackening of the turfgrass, which was exacerbated on treatments not receiving nitrogen additions. This has been described as foliar toxicity (Yust et al., 1984; Wehner, 1992). It has been reported that on most turfgrasses, rates up to 5.6 Kg Fe ha^{-1} will not result in foliar burn

(Carrow et al., 2001) although Yust (1984) reported no injury up to the 17.7 Kg Fe ha^{-1} rate. The highest rate used in this trial (97.65 Kg $FeSO4$ ha^{-1}) corresponded to a rate of 19.5 Kg Fe ha^{-1} and often resulted in blackening that was detrimental to the color quality and turf quality ratings. The water carrier volume in this trial was set at 814 L ha^{-1} in order to mimic volumes commonly used by turfgrass professionals for fungicide applications of foliar diseases (Latin, 2011). It is speculated that a larger carrier volume would decrease the blackening effect caused by the iron sulfate additions.

While some iron sulfate treatments in this study did suppress disease to an acceptable level, often turf quality was observed to be unacceptable. The primary factor responsible for decreasing turfgrass quality rating of plots with no disease was loss of turfgrass density. Experiments in the greenhouse have shown that iron applications at rates as low as 7.6 Kg Fe ha^{-1} reduced annual bluegrass shoot growth (Xu and Mancino 2001). Field studies on Kentucky bluegrass (*Poa pratensis* L.) have shown that iron phytotoxicity occurs with applications above 17.7 Kg Fe ha^{-1} (Yust et al., 1984). This could explain the loss of turf density observed in our study where iron applications ranged from 10.9 to 19.5 Kg Fe ha^{-1} $2wks^{-1}$.

Soil tests taken at the end of the trial years provide some insight into long-term effects on soil nutrient levels and particularly on soil acidity and sulfate levels. At the end of the first year of the study, soil acidity, measured as pH, was shown to be higher in plots receiving nitrogen additions in the form of urea. This is surprising as urea is known to contribute to soil acidity (Goss et al., 1977; Schroder et al., 2011). This result was only observed in the first year of the study and the difference between the lowest average pH observed (5.75) and the highest (5.85) does not likely provide any practical impact from the turf manager's perspective. Even though iron sulfate additions did not affect pH the first year of the study, differences were observed in year two. In the second year of the trial, the highest iron sulfate rates (97.65 Kg FeSo ha^{-1}) resulted in the lowest pH. Iron sulfate produces hydrogen ions as it reacts with water reducing the soil pH (Carrow et al., 2001). Even though the lower pH has been linked to less Microdochium patch incidence (Smith, 1958), turfgrass managers are encouraged to use caution when lowering the soil pH to manage Microdochium patch as the turfgrass disease anthracnose (*Colletotrichum cereale*) is suspected of being more active on annual bluegrass at a lower soil acidity (Schmid et al., 2014). Turfgrass managers will need to consider balancing fertility practices that lower soil acidity with lime additions (Kowalewski et al., 2015).

Regarding iron levels in the soil, a significant difference was only observed the first year. It was shown that treatments not receiving nitrogen resulted in the highest levels of iron. At the conclusion of the first year of the trial, these same nitrogen treatments possessed the lowest soil acidity. Even though the differences of pH and iron levels among the nitrogen treatments would not be considered large, iron is more readily available in soils possessing a lower pH (Brady and Weil, 1999), which may have contributed to the higher iron soil test results. Contrary to sulfur levels, which increased based on levels of iron sulfate applications, it is unclear why iron levels were not affected by the iron sulfate treatments in this study. One possible reason might be that the iron was bound in the upper organic matter and because this section of the soil samples was removed, the iron was not detected.

Iron is also more soluble when it is in the reduced form that is often associated with oxygen poor environments (Carrow et al., 2001). Perhaps iron that moved past the thatch layer was reduced due to saturated conditions caused by limited drainage and precipitated through the sand profile below soil testing depths. This may be a concern for turfgrass managers on areas such as golf course putting greens, where sand is placed over an oxygen-rich gravel layer. Studies have shown that precipitated iron can become oxidized at the gravel layer and compromise the drainage system (Obear and Soldat, 2014).

Conclusion

This study suggests that a low rate of nitrogen (4.88 Kg N ha^{-1}) applied every two weeks in the form of urea on annual bluegrass putting greens during the winter period does not increase Microdochium patch incidence. This information should provide management options for turfgrass managers looking to assist annual bluegrass wear tolerance and recuperation from golfer traffic during the winter months on putting greens in the Pacific Northwest without adversely affecting the risk of Microdochium patch disease. In comparison to the 4.88 Kg N ha^{-1} rate, twice the amount of nitrogen (9.76 Kg N ha^{-1}) provided negative effects on Microdochium patch incidence perhaps indicating a nitrogen threshold of 4.88 Kg N ha^{-1} every two weeks for Microdochium patch incidence. This study has also shown that as rates of iron sulfate increase, Microdochium patch incidence decreases on an annual bluegrass putting green, with the highest rate (97.65 Kg ha-1) resulting in ≤ 5% disease at the peak of disease during both years of the study. When interactions were observed, bi-weekly rates of iron sulfate applied at 97.65 Kg FeSO4 ha^{-1} in combination with 4.88 Kg N ha^{-1} was shown to decrease Microdochium patch incidence to levels acceptable for golf course putting greens, while providing nitrogen for turfgrass recuperation, however turfgrass quality ratings were not deemed acceptable due to a loss of turfgrass density and/or unacceptable turfgrass color. It is unclear as to the mode of action of iron sulfate in regards to disease suppression. Future studies will be necessary to determine if iron sulfate suppresses Microdochium patch due to iron, sulfate, a pH affect or some other factor. Other studies researching carrier volumes and application timing may result in more precise information regarding the potential of iron sulfate to inhibit Microdochium patch without a detriment to turfgrass or soil quality.

Figures:

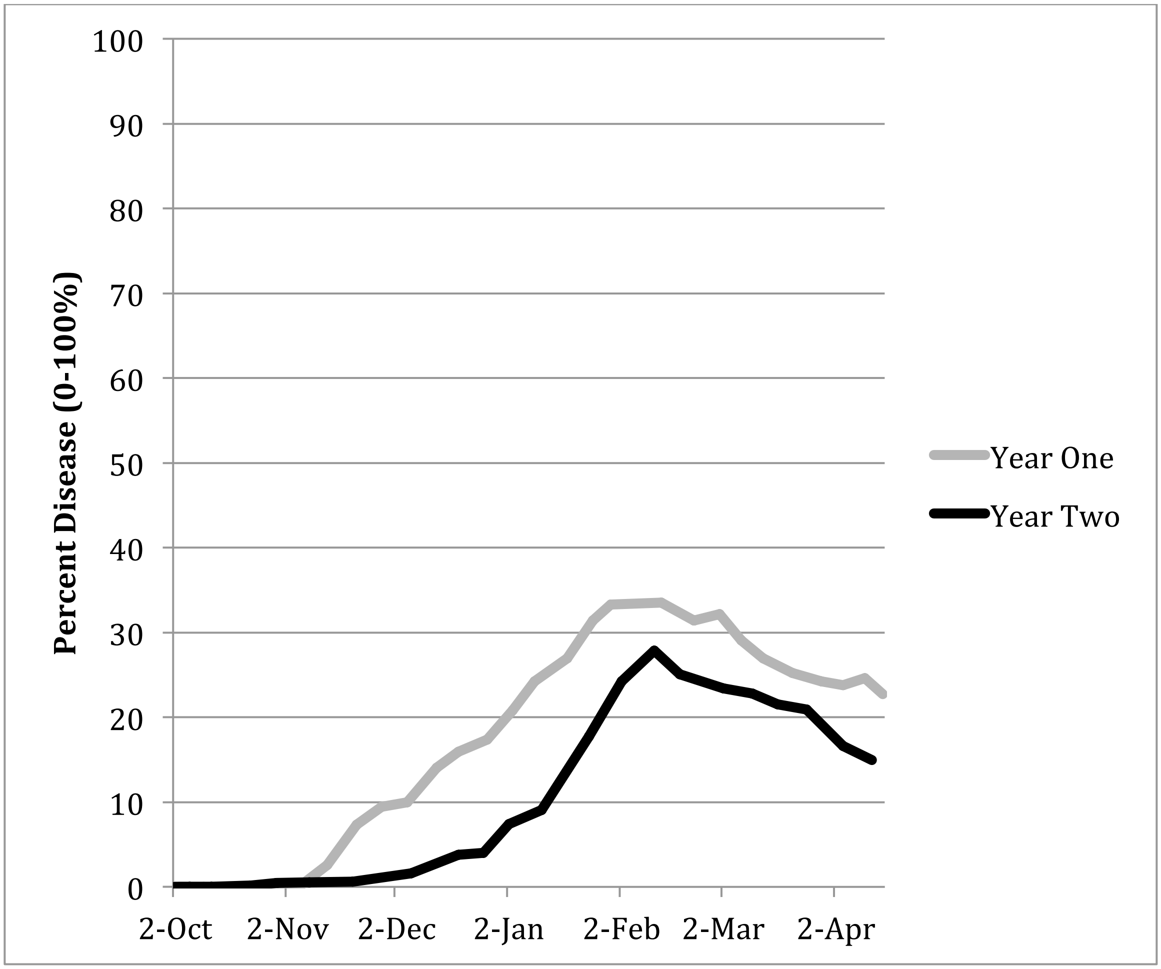

Figure 3.1: Microdochium patch development over time on an annual bluegrass putting green in Corvallis, OR over two years. Disease development over time measured as total combined disease on all plots from the onset of disease to the conclusion of the experiments for year one (1st of October 2013 to the 15th of April 2014) and year two (1st of October 2014 to the 15th of April 2015) of a two-year experiment in Corvallis, OR.

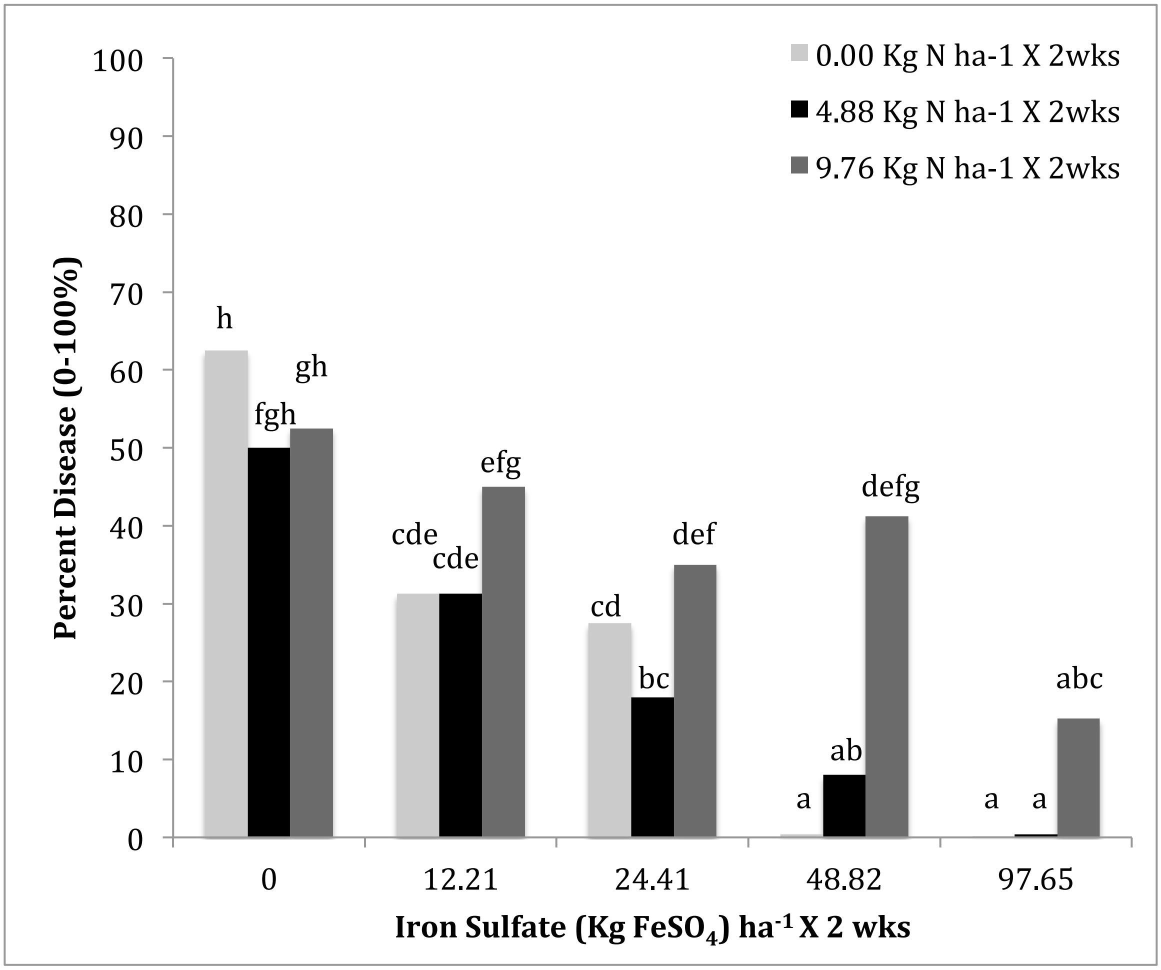

Figure 3.2: Effects of nitrogen and iron sulfate on percent disease on an annual bluegrass putting green in Corvallis, OR on February 11, 2015. Nitrogen (0.00, 4.88 and 9.76 Kg N ha^{-1}) and iron sulfate (0.00, 12.21, 24.41, 48.82, and 97.65 Kg $FeSO_4$ ha^{-1}) were applied every two weeks. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at $P \leq 0.05$. Means represent 4 data points over 4 replications.

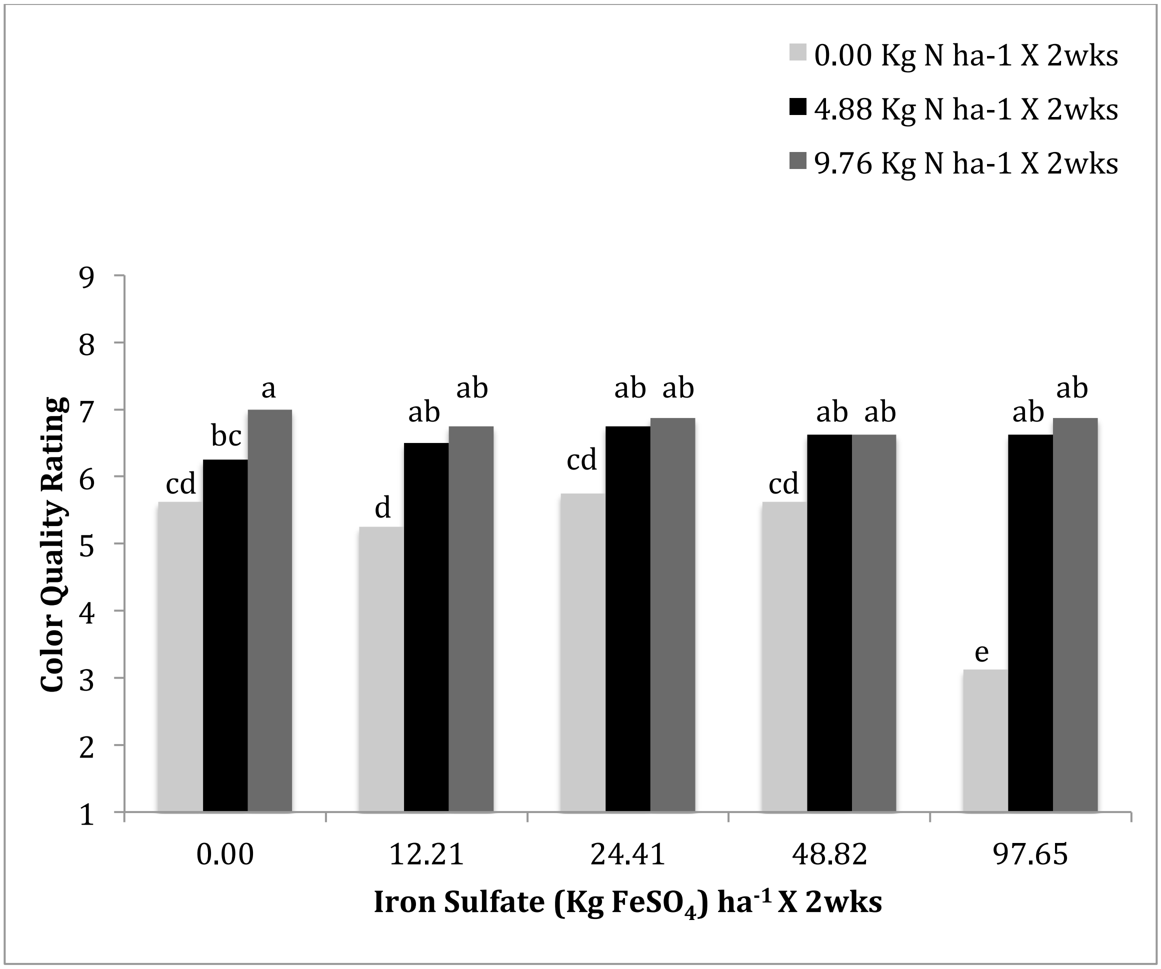

Figure 3.3: Effects of nitrogen and iron sulfate on color quality ratings on an annual bluegrass putting green in Corvallis, OR on February 13, 2014. Nitrogen (0.00, 4.88 and 9.76 Kg N ha^{-1}) and iron sulfate (0.00, 12.21, 24.41, 48.82, and 97.65 Kg $FeSO_4$ ha^{-1}) were applied every two weeks. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at $P \leq 0.05$. Means represent 4 data points over 4 replications.

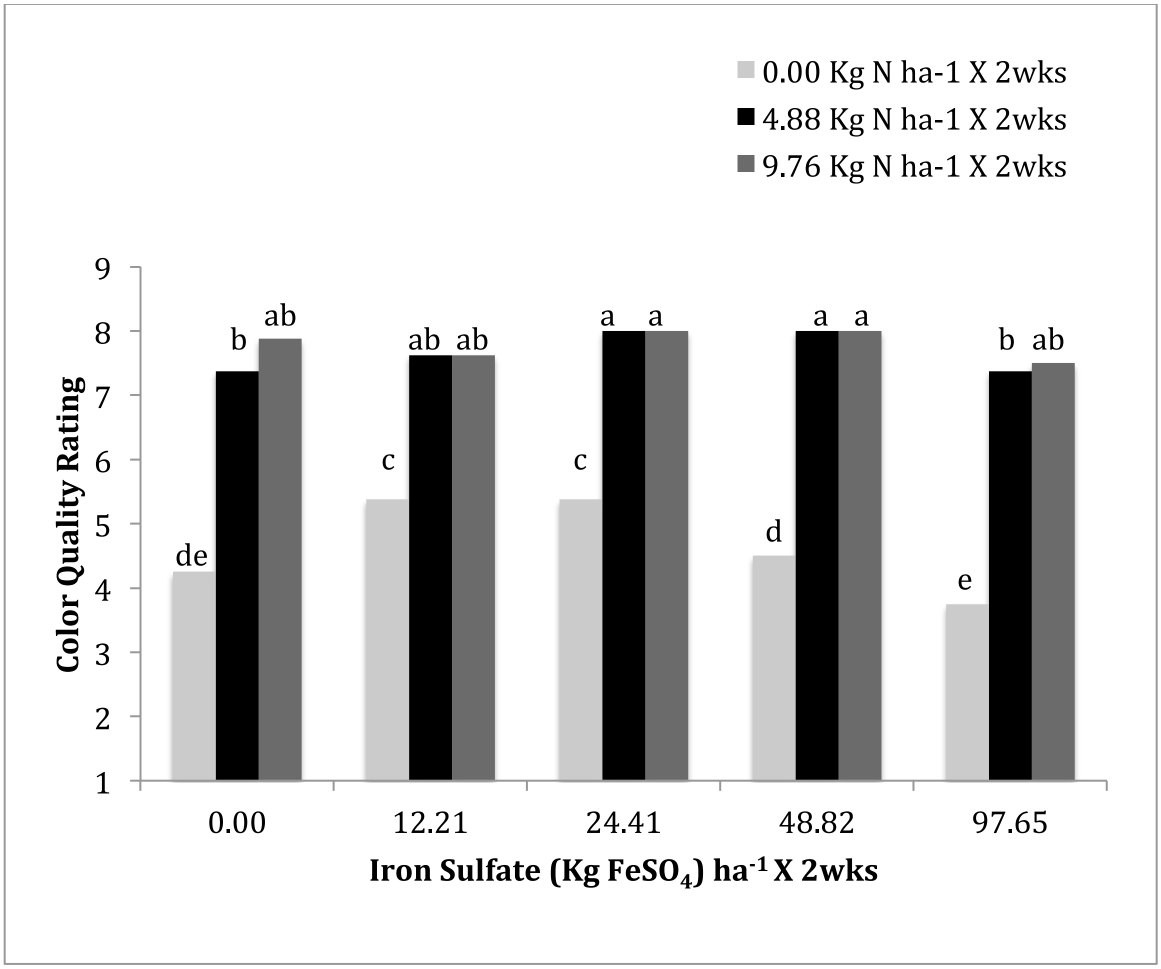

Figure 3.4: Effects of nitrogen and iron sulfate on color quality ratings on an annual bluegrass putting green in Corvallis, OR on February 11, 2015. Nitrogen (0.00, 4.88 and 9.76 Kg N ha^{-1}) and iron sulfate (0.00, 12.21, 24.41, 48.82, and 97.65 Kg $FeSO_4$ ha^{-1}) were applied every two weeks. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at $P \leq 0.05$. Means represent 4 data points over 4 replications.

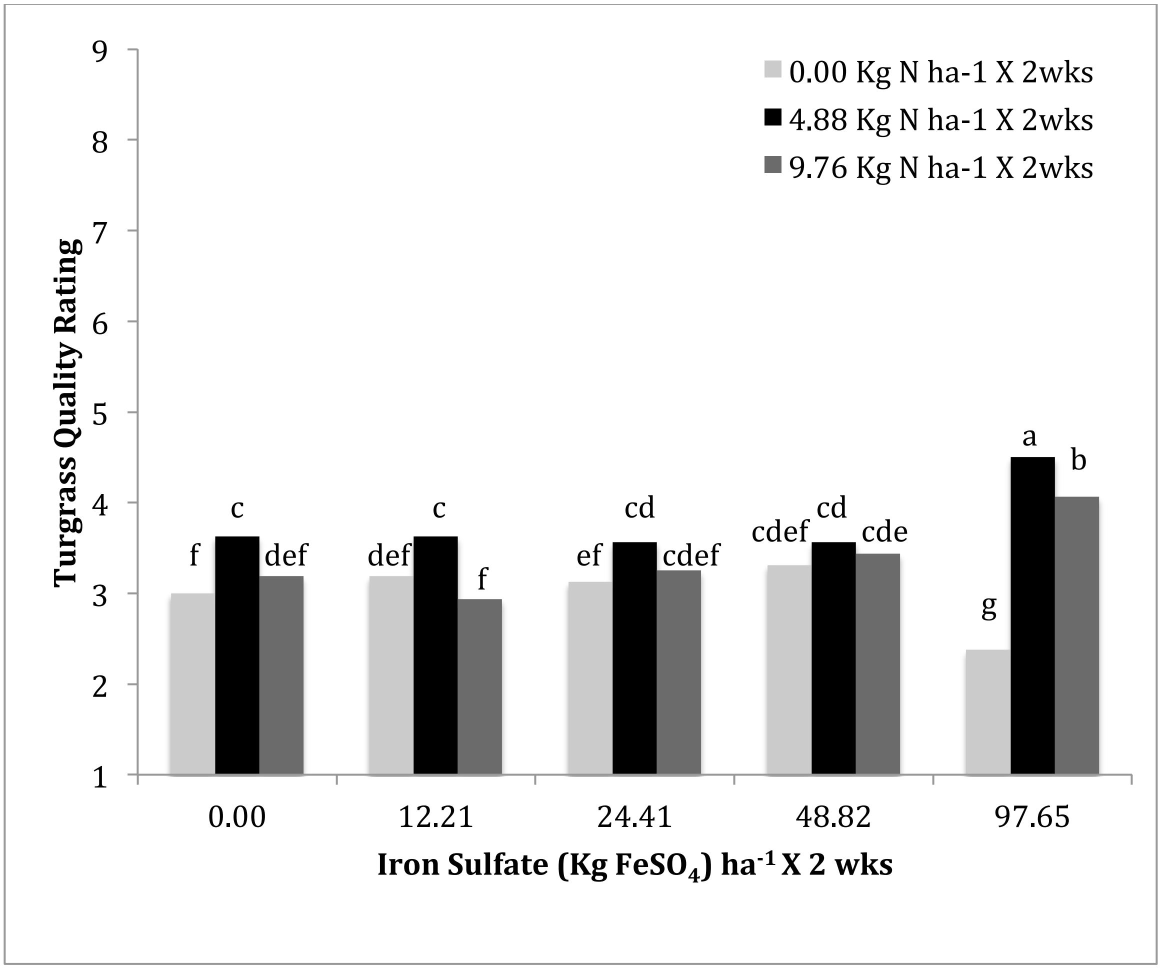

Figure 3.5: Effects of nitrogen and iron sulfate on turfgrass quality ratings on an annual bluegrass putting green in Corvallis, OR on February 13, 2014. Nitrogen (0.00, 4.88 and 9.76 Kg N ha^{-1}) and iron sulfate (0.00, 12.21, 24.41, 48.82, and 97.65 Kg FeSO$_4$ ha^{-1}) were applied every two weeks. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at P≤ 0.05. Means represent 4 data points over 4 replications.

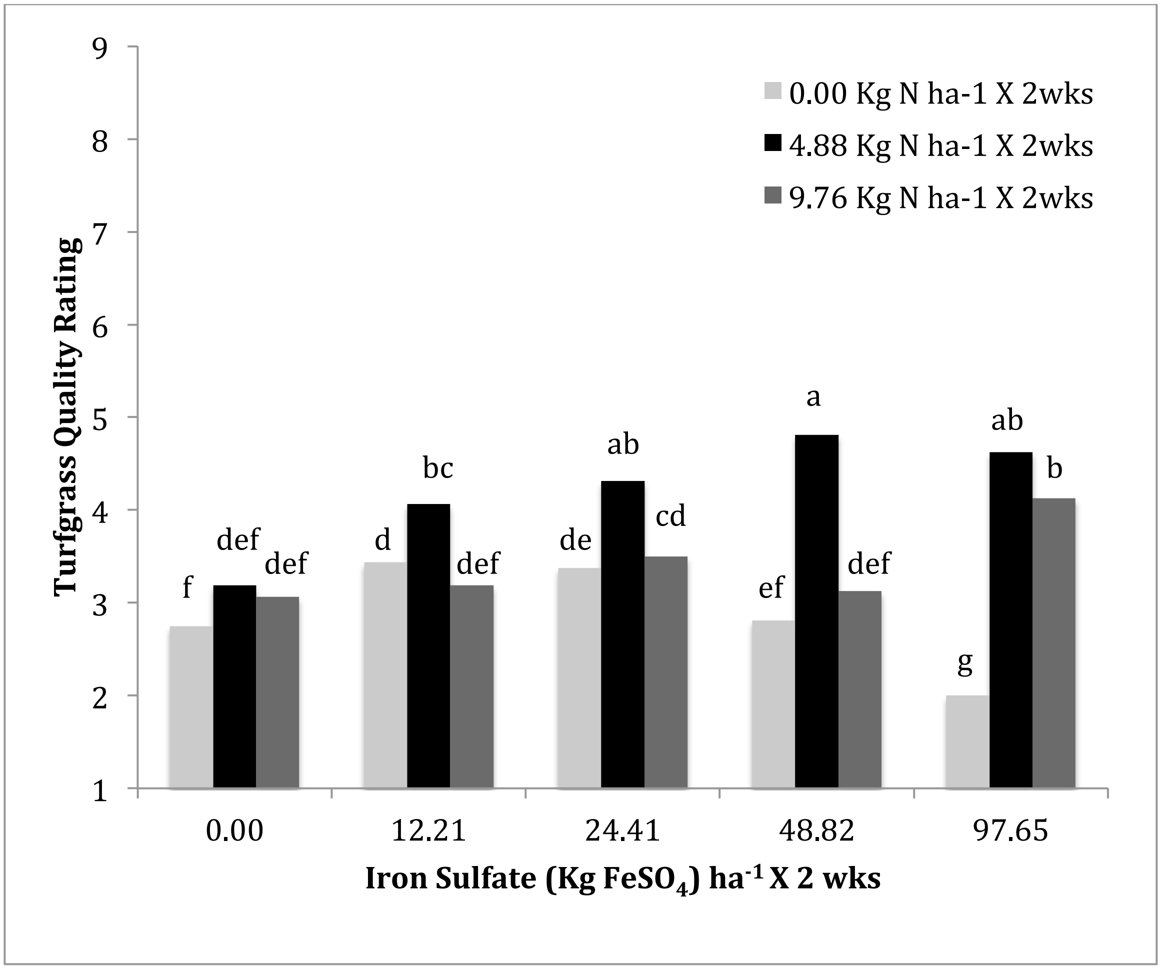

Figure 3.6: Effects of nitrogen and iron sulfate on turfgrass quality ratings on an annual bluegrass putting green in Corvallis, OR on February 11, 2015. Nitrogen (0.00, 4.88 and 9.76 Kg N ha^{-1}) and iron sulfate (0.00, 12.21, 24.41, 48.82, and 97.65 Kg $FeSO_4$ ha^{-1}) were applied every two weeks. Means denoted with the same letter are not significantly different according to Fisher's protected least significant difference test at $P \leq 0.05$. Means represent 4 data points over 4 replications.

Tables

Table 3.1. Analysis of variance results for percent Microdochium patch (*Microdochium nivale*) disease, area under disease progress curve, turf color, turf quality, soil test pH, S and Fe for two different years on an annual bluegrass putting green as influenced by nitrogen and iron sulfate treatments in Corvallis, OR over two years.

Year One

Source Of Variation	DF	Perc. Dis.	AUDPC	Turf Color	Turf Quality	Soil Test pH	S	Fe
		--------------------	Pr > F	------------------				
Nitrogen (N)	2	*	*	***	***	*	ns	**
Iron Sulfate ($FeSO_4$)	4	***	***	***	*	ns	**	ns
N*$FeSO_4$	8	ns	ns	***	***	ns	ns	ns

Year Two

Source Of Variation	DF	Perc. Dis.	AUDPC	Turf Color	Turf Quality	Soil Test pH	S	Fe
		--------------------	Pr > F	------------------				
Nitrogen (N)	2	***	***	***	***	ns	ns	ns
Iron Sulfate ($FeSO_4$)	4	***	***	***	***	*	***	ns
N*$FeSO_4$	8	*	ns	**	***	ns	ns	ns

*** Significant at a 0.001 level of probability
** Significant at a 0.01 level of probability
* Significant at a 0.05 level of probability
ns Not significant at a 0.05 level of probability

Table 3.2. Area under disease progress curve and percent Microdochium patch (*Microdochium nivale*) disease and soil test results for pH, S and Fe for two different years on an annual bluegrass putting green as influenced by nitrogen and iron sulfate treatments in Corvallis, OR over two years.

Year One[z]	AUDPC[y]		Percent Disease[x]		Fe[w]		S[w]		pH[w]	
0.00 N[v]	16.2	b[u]	29.4 %	b	53.9	a	13.2	ns	5.75	b
4.88 N	15.0	b	29.4 %	b	47.5	b	11.1	ns	5.82	ab
9.76 N	24.0	a	42.0 %	a	46.2	b	10.7	ns	5.85	a
00.00 $FeSO_4$[t]	28.5	ab	57.5 %	a	49.2	ns	9.2	b	5.80	ns
12.21 $FeSO_4$	29.7	a	48.8 %	ab	47.8	ns	10.3	b	5.78	ns
24.41 $FeSO_4$	19.2	bc	37.9 %	b	47.3	ns	11.0	b	5.83	ns
48.82 $FeSO_4$	14.0	c	22.5 %	c	50.3	ns	11.4	b	5.79	ns
97.65 $FeSO_4$	0.7	d	1.3 %	d	51.3	ns	16.4	a	5.83	ns

Year Two[s]	AUDPC[r]		Percent Disease[q]		Fe[p]		S[p]		pH	
0.00 N	6.0	b	24.4 %	b	67.9	ns	13.0	ns	5.61	ns
4.88 N	3.3	b	21.5 %	b	64.0	ns	10.7	ns	5.59	ns
9.76 N	10.9	a	37.8 %	a	66.8	ns	10.5	ns	5.63	ns
00.00 $FeSO_4$	13.6	a	55.0 %	a	65.3	ns	6.4	d	5.66	a
12.21 $FeSO_4$	7.6	b	35.8 %	b	66.6	ns	7.4	cd	5.64	a
24.41 $FeSO_4$	6.0	bc	26.8 %	b	61.4	ns	10.1	bc	5.66	a
48.82 $FeSO_4$	4.0	bc	16.5 %	c	67.4	ns	12.1	b	5.58	ab
97.65 $FeSO_4$	2.5	c	5.3 %	d	70.5	ns	21.0	a	5.51	b

[z] Year one trial began on 26 September 2013 and concluded on 12 April 2014.

[y] AUDPC (Area under disease progress curve) data was calculated from the beginning of the year one trial (26 September 2013) until the peak of disease (13 February 2014).

[x] Percent disease data was analyzed for the peak of disease of the first trial year (13 February 2014).

[v] N=Kilograms of nitrogen per hectare applied every two weeks.

[u] Means in the same column followed by the same letter are not significantly different according to Fisher's protected least significant difference ($P \leq .05$).

[t] FeSO4= Kilograms of iron sulfate per hectare applied every two weeks.

[s] Year two trial began on 22 September 2013 and concluded on 12 April 2015.

[r] AUDPC (Area under disease progress curve) data was calculated from the beginning of the year one trial (22 September 2014) until the peak of disease (11 February 2015).

[q] Percent disease data was analyzed for the peak of disease of the second trial year (11 February 2015).

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SUMMARY

Three two-year field trials were performed with the objective to explore and quantify possible means of controlling Microdochium patch in the absence of traditional fungicides. In the first experiment, evidence was provided that showed rolling treatments were able to reduce Microdochium patch intensity on annual bluegrass putting greens, although the effects were only observed in the absence of other disease control techniques such as Civitas One, Sulfur DF or PK Plus combinations. In spite of the intensity of Microdochium patch disease observed in this study, suppression of Microdochium patch through the use of alternative controls to levels acceptable for golf course putting greens was observed for multiple treatment combinations. Repeated applications of the mineral oil Civitas One suppressed Microdochium patch incidence to levels < 1.5% in both years of this two year study. However, abiotic damage was observed when rolling was combined with Civitas One treatments therefore caution is advised for turfgrass managers when using Civitas One bi-weekly throughout the winter period. Further research is warranted regarding application rates and timings in order to determine a safe use of this product for golf course putting green applications. Sulfur DF combined with PK Plus was also able to reduce Microdochium patch although to less than 1%. These results suggest an additive effect of this fertility treatment combination concerning Microdochium patch control. In addition, abiotic damage was not observed in the Sulfur DF + PK Plus treatments, therefore this combination provides an IPM tool that would assist turfgrass managers in managing Microdochium patch in the absence of traditional fungicides. Further research is warranted to better understand the mechanisms involved in the additive effect of Microdochium patch control with Sulfur DF and PK Plus combinations.

In the second experiment, year one revealed that the combination of rolling and biological control products BW136N and Rhapsody was shown to reduce Microdochium patch disease. In the second year, rolling suppressed Microdochium patch incidence compared to plots not receiving the rolling treatments, and BW136N as well as Rhapsody suppressed Microdochium patch disease. Even though the treatments used in this study did not provide results equivalent to those often obtained from traditional fungicide products, it can be speculated that if future legal constraints result in chemical disease control no longer being an option, even the limited disease control observed in this study would be considered superior to no control at all. Future research may also find that rolling or biological controls such as BW136N or Rhapsody might be used in combination or in rotation with other disease management practices such as chemical controls in order to decrease the overall use of fungicides.

In the third experiment, there was suggestion that a low rate of nitrogen (4.88 Kg N ha^{-1}) applied every two weeks in the form of urea on annual bluegrass putting greens during the winter period does not increase Microdochium patch incidence. This information should provide management options for turfgrass managers looking to assist annual bluegrass wear tolerance and recuperation from golfer traffic during the winter months on putting greens in the Pacific Northwest without adversely affecting the risk of Microdochium patch disease. In comparison to the 4.88 Kg N ha^{-1} rate, twice the amount of nitrogen (9.76 Kg N ha^{-1}) provided negative effects on Microdochium patch incidence perhaps indicating a nitrogen threshold of 4.88 Kg N ha^{-1} every two weeks for Microdochium patch incidence. This study also identified a direct correlation between iron sulfate rates and Microdochium patch incidence, with the highest rate (97.65 Kg ha-1) resulting in ≤ 5% disease. When interactions were observed, bi-weekly rates of iron sulfate applied at 97.65 Kg FeSO4 ha^{-1} in combination with 4.88 Kg N ha^{-1} was shown to decrease Microdochium patch incidence to levels acceptable for golf course putting greens, while providing nitrogen for turfgrass recuperation, however turfgrass quality ratings were not deemed acceptable due to a loss of turfgrass density and/or unacceptable turfgrass color. It is unclear as to the mode of action of iron sulfate in regards to disease suppression. Future studies will be necessary to determine if iron sulfate suppresses Microdochium patch due to iron, sulfate, a pH affect or some other factor. Other studies researching carrier volumes and application timing may result in more precise information regarding the potential of iron sulfate to inhibit Microdochium patch without a detriment to turfgrass or soil quality.

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