# The Effect of Ferrous Sulfate on Annual Bluegrass, Silvery Thread Moss, and Dollar Spot Populations Colonizing Creeping Bentgrass Putting Greens

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## ABSTRACT

Annual bluegrass (*Poa annu*a L.) is the most problematic weed to control in creeping bentgrass (*Agrostis stolonifera* L.) putting greens. The objective of this study was to transition a mixed putting green stand of annual bluegrass and creeping bentgrass to a monoculture by using fertilizers and plant growth regulators that selectively inhibit annual bluegrass. A 25 year old loamy sand rootzone research green, planted with 'Penn-Eagle' creeping bentgrass, with roughly 45% initial annual bluegrass coverage was utilized. The biweekly application of ammonium sulfate (4.8 kg ha$^{-1}$) with treatments of ferrous sulfate at rates of 0, 12.2, 24.4, and 48.8 kg ha$^{-1}$ and in combination with seaweed extract (12.8 L ha$^{-1}$) or paclobutrazol (0.37 L ai ha$^{-1}$ spring and fall; 0.18 L ai ha$^{-1}$ summer) were applied March to October, 2011 and 2012. Plots receiving the highest rate of ferrous sulfate resulted in annual bluegrass infestation declines from an early trial amount of 45% to a final average of 20% but also resulted in unacceptable late-summer events of annual bluegrass collapse. The ferrous sulfate medium rate resulted in a smooth transition from early-trial annual bluegrass infestation of 45% to an end of trial infestation of 20% and had the highest putting green quality. Previous research has reported that consistent use of paclobutrazol can effectively and safely reduce annual bluegrass infestations. In this trial annual bluegrass was reduced to 9% infestation after three months of application. Two unexpected observations from this trial were that ferrous sulfate, applied at medium to high rates, significantly reduced silvery thread moss (*Bryum argentum* Hedw.) populations and occurrences of dollar spot (*Sclerotinia homoeocarpa* F. T. Bennett) disease. Dollar spot control with ferrous sulfate has not previously been reported in the literature, so additional studies were designed to investigate this phenomenon further. A creeping bentgrass putting green study was conducted to determine if sulfur, iron, or the two combined as ferrous sulfate decreases dollar spot activity. Ferrous sulfate resulted in the highest turf quality and suppressed *S. homoeocarpa* infection, even during high disease pressure. Fe-EDTA suppressed dollar spot infection as well as ferrous sulfate but quality declined to unacceptable levels during the summer, due to Fe-EDTA only. Sulfur did not affect or increased *S. homoeocarpa* infection, indicating that a high and frequent foliar rate of iron is responsible for dollar spot control. An *in-vitro* study was conducted to determine if agar pH in combination with iron concentrations affects mycelial growth of *S. homoeocarpa*. Results from this trial indicated that 5.4 agar pH is an optimal pH for mycelial growth. The 10 to 100 mg iron kg$^{-1}$ concentration had little effect on mycelial growth at 5.0 and 5.5 pH, but increased growth at 4.5 and 6.5 pH. As the iron concentration was increased from 10 to 100 to 1000 mg kg$^{-1}$, mycelial growth decreased or stopped. Our final conclusions are that seasonal biweekly foliar applications of the medium rate of ferrous sulfate (24.4 kg $ha^{-1}$) safely and effectively reduced annual bluegrass infestation out of a creeping bentgrass putting green, while also effectively suppressing silvery thread moss and dollar spot incidence.

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# The Influence of Ferrous Sulfate, Seaweed Extract, and Paclobutrazol on Annual Bluegrass and Silvery Thread Moss Colonizing Creeping Bentgrass Putting Greens

## Introduction

Creeping bentgrass (*Agrostis stolonifera* L.) provides an ideal putting surface for golf courses located in temperate climates because it provides a uniform playing surface, moderately tolerates heat and disease pressure, has a medium irrigation requirement, and is aesthetically pleasing (Emmons, 2008). Monocultures of creeping bentgrass greens are invaded by annual bluegrass (*Poa annua* L.) and silvery thread moss (*Bryum argenteum* Hedw.), which can be difficult to consistently eliminate. Both species disrupt putting green uniformity, causing an unacceptable playing surface.

Annual bluegrass disrupts putting surface uniformity and lowers the aesthetic quality provided by a creeping bentgrass monoculture. Annual bluegrass causes decreases in turf quality that are attributed to fall through spring seed head inflorescence development and the light green color annual bluegrass turns during these time periods (Lyons, 2004; Hagley et al., 2002). Compared to creeping bentgrass, annual bluegrass is more susceptible to summer stresses and requires more maintenance inputs to maintain acceptable performance during the summer months. Annual bluegrass is less likely to withstand summer stresses, such as higher temperatures and disease pressure that may cause it to die and leave bare soil on the putting surface (Vargas, 2005). In order to maintain a putting green that contains a significant annual bluegrass population, and avoid unacceptable summer decline, annual bluegrass requires increased irrigation, fungicide applications, and more phosphorus and potassium fertilizer inputs (Goss et al., 1977; Piotras and Johnston, 1989; Vargas, 2005). For these reasons, most turf managers often want to eradicate existing annual bluegrass populations or prevent its colonization.

Herbicides have thus far been relatively unsuccessful in the selective control of annual bluegrass in putting greens. The use of herbicides to control annual bluegrass populations can be problematic because they often are injurious to creeping bentgrass. For example, the application of bispyribac-sodium in research trials has been observed to be effective at controlling annual bluegrass, but has also been observed to cause unacceptable injury to creeping bentgrass maintained for putting greens. In addition, bispyribac-sodium applications can cause the sudden death of existing annual bluegrass populations, creating voids of turf in the putting green, reducing playing conditions. For these reasons bispyribac-sodium is not recommended for application on creeping bentgrass putting greens (McCullough and Hart, 2010; Teuton et al., 2007).

In recent years, silvery thread moss has become a common occurrence on creeping bentgrass putting greens as a result of reduced fertility and lower mowing heights. Superintendents often employ these turf management practices to meet rising player expectations for faster and more uniform putting surfaces. These practices decrease bentgrass competiveness with silvery thread moss and increase its colonization into the putting green. Moss infestations lower putting green quality and playability. The restriction of mercury-based fungicides, which effectively controlled silvery thread moss, removed one of the most common chemical approaches for its control in putting green turf (Turgeon et al., 2009). Few herbicides, such as carfentrazone, have been registered for silvery thread moss control in creeping bentgrass putting greens (Borst et al., 2010; Kennelly et al., 2010). Even those herbicides that have been used for suppression or control can be costly to frequently apply or possibly cause injury to the desirable creeping bentgrass.

## Literature Review

Given its botanical name in 1753 by Linnaeus, *Poa annua* L. has been observed in Europe, North Africa, North Asia, Australia, New Zealand, North and South America, and within the Arctic Circle. At many of these locations, annual bluegrass has been found to thrive in association with human habitation and frequent disturbance (Peel, 1982). Subject to frequent disturbance from normal golf play, mismanagement, and environmental stresses that thin turf stands, golf courses are ideal locations for large annual bluegrass populations to survive. The ability of annual bluegrass to adapt to the various environmental and maintenance conditions on golf courses has resulted in a difference in texture, density, and flowering time amongst various populations across the same golf course (Johnson, 1993). As a result of such differential selection pressure, two biotypes are commonly observed and they are referred to as annual and perennial biotypes. There are a number of differences between the two biotypes with respect to their growth habit, leaf morphology, tiller density, rooting, vegetative phase duration, seed production, and summer stress tolerance.

The low mowing heights (2.5 to 4 mm) imposed on intensively managed putting greens creates an environment favoring grasses with a creeping growth habit. The growth habit of the different annual bluegrass biotypes reflects the environment in which each survives. The perennial biotype has been observed to have a more prostrate, creeping growth habit, which is preferred for lower mowed putting greens (Gibeault, 1974). Described as having a more erect growth habit, the annual biotype is more prevalent in roughs and fairways that are maintained at mowing heights of 12 mm or higher. Despite low mowing heights of putting greens, the erect annual biotype is temporarily able to survive in these areas, but they eventually become dominated by the perennial biotype (Cordukes, 1977; Hovin, 1957).

Leaf morphology and tiller density characteristics are often used to distinguish the two biotypes from each other. For example, leaf texture of the annual biotype has been observed to be wider than the perennial (Hagley et al., 2002). The upright annual biotype produces fewer tillers than the prostrate perennial biotype, resulting in the perennial having a dense, compact growth habit (Cordukes, 1977; Hovin, 1957). The higher tiller density increases the perennial biotype's ability to spread and fill in disturbed areas on putting greens (Hovin, 1957).

The weather conditions required for flowering and flowering time varies between the two biotypes. The annual biotype flowers between fall and spring, while the perennial flowers mainly in the spring (Hagley et al., 2002). The annual biotype produces panicles at 51 days after first occurrence of night temperatures below 8°C. The perennial biotype requires vernalization at 4 to 8°C for 8 to 12 weeks before panicle production begins (McElroy et al., 2002). The extended flowering period of the annual biotype allows it to be a prolific seed producer and to occur more commonly across mowing heights and climates (Lush, 1989; Law, 1981).

Seed production and germination timing determine which annual bluegrass biotype will survive long term on a putting green. The annual biotype produces many seeds that are dormant after being shed from the inflorescence (Gibeault, 1971; Hovin, 1957). During the spring, the perennial biotype produces seeds that immediately germinate after falling from inflorescences (Hovin, 1957; Lush, 1989). The non-dormant perennial biotype seeds have been shown to germinate during the summer months, while annual biotype seeds remained dormant and non-competitive with perennial seeds (Gibeault, 1971).

The growth and development of new annual bluegrass plants begins with a vegetative phase followed by seed production. These phases vary among the two biotypes and have an effect on how the species survives stressful environments. Annual biotypes have a short vegetative phase, putting a large share of its resources into reproduction rather than into vegetative growth (Piotras and Johnston, 1989; Lush, 1988a). Under conditions of decreased water availability, the annual biotype is still able to maintain a high density of inflorescences per unit area. This suggests that annual biotypes may be more adapted to drier conditions where more carbohydrates are partitioned to seed production rather than growth (Slavens et al., 2011). In contrast, the perennial biotype has a lower seed yield and spreads more by vegetative growth (Hovin, 1957). The perennial biotype requires more frequent inputs, such as irrigation, to survive periods of extended drought and heat stress (Gibeault, 1971).

During colonization of a new area, or under stressful environments, annual bluegrass most often behaves as an annual biotype (Lush, 1989; Law, 1981). The annual biotype's adaptability to produce a large amount of seed allows it to escape adverse environmental conditions and increase the survivability of the species (Piotras and Johnston, 1989; Johnson et al., 1993). Annual biotypes can rapidly populate and colonize, but typically die from summer heat and drought after producing abundant seeds (Gibeault, 1974).

### Pollination and Seed Production

Johnson et al. (1993) observed that annual bluegrass disperses its pollen between 3:00 and 8:00 am, allowing it to quickly and effectively self-pollinate during a period when the wind is typically the calmest. An additional benefit of self-pollination is for example, if one seed falls in an open niche the plant will be able to reproduce without other annual bluegrass plants nearby. Annual bluegrass is also able to cross pollinate as indicated by its efficiency in pollen liberation (Piotras and Johnston, 1989; Koshy, 1969). However, cross pollination depends on the right environmental conditions and on the population size of the species in a given area (Johnson et al., 1993).

Viable seeds are produced and dispersed within 24 to 48 hours after pollination occurs. Maximum seed production occurs within a 14 to 18 day period in the spring, peaking at 363-433 growing degree days (base 10°C) and ceasing after 433 growing degree days (Danneberger and Vargas, 1984). It has been estimated that a creeping bentgrass green located in a temperate zone infested with annual bluegrass can yield between 150,000 to 675,000 seeds $m^{-2}$ $y^{-1}$ (Lush, 1988a). Despite the low mowing conditions of a putting green, annual bluegrass yields large numbers of viable seeds. Even though the panicle is often excised from the plant by mowing on the same day that pollination occurs, viable seeds are still produced (Koshy, 1969).

### Soil Seed Bank

Annual bluegrass seeds are constantly produced and dispersed in the environment, eventually entering the soil seed bank. This is evident by the genetic variability observed among seeds in putting green soils, indicating that many of the seeds were transported to the green from other locations (Lush, 1988b). In the soil seed bank, annual bluegrass seeds are found in the top few centimeters and remain until the right conditions to germinate occur. When a site becomes disturbed and the above ground creeping bentgrass dies out or is weakened, annual bluegrass seeds are able to germinate and grow (Lush, 1988a). Core aerification, a common cultural practice for creeping bentgrass greens, brings buried annual bluegrass seeds to the green surface where they are able to germinate (Lush, 1988b; Kaminski and Dernoeden, 2007).

As annual bluegrass plants die or decline during the summer months, they are replaced by the germinating seeds from the seed bank or vegetative growth from annual bluegrass or creeping bentgrass. The timing of germination ensures the long term survival of the population in the putting green. The large number of seeds in the seed bank defines annual bluegrass as an opportunistic weed waiting for a disturbance to germinate and fill in the void of turf (Lush, 1988b).

The soil seed bank of a putting green located in a temperate zone of Southern Australia contains 24 different species of seeds, but only four of those species appeared on the surface of the green as mature plants. A count of the number of seeds in the seed bank found that annual bluegrass had the most seeds, with creeping bentgrass comprising the second most seeds in the soil. The amount of seeds in the seed bank varied with season. The number of annual bluegrass seeds peaked at approximately 168,000 $m^{-2}$ from late-winter to spring and declined to about 30,000 seeds $m^{-2}$ in late-summer to fall. The decline was attributed to seedling germination, indicating that most of the seeds in the seed bank are transient. Many of the seeds that are persistent in the seed bank were buried in the thatch or soil underlying the layer of current growth (Lush, 1988b).

### Environmental Conditions for Germination

Kaminski and Dernoeden (2007) recorded that bare soil is more conducive to promoting annual bluegrass seed germination than soil with an established stand of turf. Younger (1959) reported that seeds were unable to establish under a dense mat of a perennial grass. However, germination was observed across varying photoperiods indicating germination was not inhibited by complete darkness that may be created by a perennial mat of turf (McElroy et al., 2004; Lush, 1988a). Germination was most abundant in late-summer to early-fall and decreased into late-fall, early-winter. An increase in seedling emergence was again observed in late-winter and early-spring, but would soon slow again during the summer months (Gibeault, 1974; Kaminski and Dernoeden, 2007). Kaminski and Dernoeden (2007) observed annual bluegrass seedling emergence in Maryland occurring between late September and the first two weeks of October. During this time 76% of annual bluegrass seed germination occurred, while the remaining 24% germinated over the next seven months. Seasonal seedling emergence timing varied due to differences in air (mean 20°C) and soil (mean 15°C) temperature across the years.

## Genetic Variability and Adaptation

Adams and Bryan (1977) collected various annual bluegrass samples from different sports fields across the United Kingdom and observed genetic variability among the different samples. They concluded that variation in sports turf maintenance over a period of time brings about the selection of annual bluegrass types suited for that particular environment. Annual bluegrass collected from a putting green was found to produce more tillers when compared with annual bluegrass collected from other sports fields. The authors concluded that annual bluegrass populations grew rapidly in terms of vertical growth on soccer and other sport fields as opposed to golf and bowling greens (Adams and Bryan, 1977).

Perennial biotypes that are left unmowed produce annual bluegrass seedlings that are observed to be annual biotypes (Mantia and Huff, 2011). This observation indicates that the perennial biotype is unstable and may be regulated by an epigenetic mechanism. The authors hypothesize that mowing acts as a stress that induces the epigenetic mechanism to silence a gene regulating plant growth in an effort to escape excessive wounding from the frequent, low mowing of putting greens (Mantia and Huff, 2011).

## Invasion and Colonization of Creeping Bentgrass Putting Greens

Described as a rapidly growing grass that can germinate, grow, and produce viable seed within one month, annual bluegrass is thought to be naturally distributed (Ferguson, 1936). The ability to distribute itself widely across a location led to the conclusion that putting green-adapted populations may have come from annual biotypes located in the rough or other locations (Lush, 1989). Once a seed reaches a putting green, the environment is favorable to the species and the biological adaptations of the species allow it to invade and colonize the putting green surface area (Piotras and Johnston, 1989). Annual bluegrass starts out as an annual biotype and then the population soon becomes more perennial in nature. This observation is due to the interbreeding and selection, caused by frequent low mowing, for the prostrate morphology of the perennial biotype in a putting green. Older golf greens often display an established and stable perennial biotype, while newer putting greens display an unstable annual biotype that is still in the initial stages of putting green colonization (Hagley et al., 2002).

Colonization, population growth, and maturity of an annual bluegrass population into a newly disturbed site were observed by Law (1981). Germination and growth began in early-spring and increased steadily until the population was quadrupled in a three month period, reaching a population maximum. Fluctuations in population size occurred within the annual bluegrass stand as the seasons changed, with increases in spring and fall, and decreases in winter and summer (Law, 1981).

### Seasonal Growth and Competition with Creeping Bentgrass

A creeping bentgrass putting green located in a temperate zone of Southern Australia had five to six different species growing throughout the year, with annual bluegrass and creeping bentgrass present year round. These two species are constantly competing to survive in the same environment that can favor the growth of one over the other at various times of the year. Reproduction and vegetative growth determines how well each species survives and competes on a putting green. Creeping bentgrass reproduction only occurs vegetatively, an attribute that implies better adaptation to undisturbed sites. Annual bluegrass spreads by many seeds and vegetative growth, attributes that imply better adaptation to disturbed sites like a putting green (Lush, 1988a).

Samples taken from a putting green located in a temperate climate of Australia were observed to contain mostly creeping bentgrass, with a few germinated annual bluegrass seedlings, during the summer months. The fewer germinated seedlings may indicate that in the summer creeping bentgrass is producing more tillers, while the flowering annual bluegrass is thinning out and dying. Through the summer months, the annual biotype dies out and the perennial biotype survives, maintaining the annual bluegrass population on the green. The annual bluegrass population may also be maintained by the annual biotype whose generations overlap during the summer months (Lush, 1988a).

During a trial to observe the different characteristics between creeping bentgrass and annual bluegrass, a mixing of the species occurred with an annual bluegrass invasion into the creeping bentgrass. 'Penn A-4' creeping bentgrass was found to be more resistant to annual bluegrass invasion compared to 'Penncross' because of its greater tiller density as compared to older, less dense cultivars. When compared with creeping bentgrass, annual bluegrass often has greater tiller density, allowing the plant to better expand into disturbed areas through increased vegetative propagation (Lyons, 2004).

Lush (1988a) has noted that tiller density for each species changes throughout the year. By late-fall and through the winter, annual bluegrass tiller density exceeded that of creeping bentgrass, allowing it to become the main component of the green. In early-spring, creeping bentgrass increased its tiller density and competed favorably with the flowering annual bluegrass to become the dominant component of the putting green by the end of spring.

The varying root depths between the species influences tolerance to abiotic and biotic stresses. Annual bluegrass has been shown to persist better than creeping bentgrass in compacted and excessively wet soils because of its shallower root system. During periods of drought, the shallow root system is not able to reach deeper into the soil for moisture, leading to annual bluegrass decline. Comparing the two species, creeping bentgrass had a deeper rooting system than an annual bluegrass perennial biotype that had 75 to 85 % of its root mass in the top 3 cm (Lyons, 2004).

Extremely hot weather can put both grass species under stress. As air temperature increases, soil temperatures increase causing stress on both root systems and above ground plant structures. As temperature increases during the summer months (> 30°C), annual bluegrass is unable to tolerate the heat and begins to decline in health and viability (Gibeault, 1974). When annual bluegrass varieties were exposed to 100% relative humidity and 47°C for 2.5 hours, damage could be seen immediately after the treatment began (Cordukes, 1977). When compared with creeping bentgrass during heat and water stress, annual bluegrass will wilt before creeping bentgrass, indicating a poorer performance during drought and heat stress (Vargas, 2005).

### Soil pH, Nutrients, and Nutrient Availability at Various pH Levels

During the mid-1930s the theory of acidifying the soil was put into practice to control annual bluegrass. After the acid theory was put into practice, a series of droughts occurred. Because of the lack of irrigation systems, the droughts caused loss in turf stands and were blamed on the acid theory practice (Arthur, 2003). The practice of acidifying soil is known to reduce optimum growth even though the turf can tolerate the low soil pH. Creeping bentgrass can grow and persist at a pH of 5.0, while annual bluegrass is less competitive (Carrow et al., 2001).

Juska and Hanson (1969) observed the effects of soil pH (4.5 and 6.5) and fertilizer (Nitrogen at 0 or 148 kg $ha^{-1}$, Phosphorus at 0 or 188 kg $ha^{-1}$, Potassium at 0 or 168 kg $ha^{-1}$) applications on annual bluegrass growing on loamy sand. A decrease in clipping and root yields was observed at a 4.5 pH compared to the 6.5 pH. The application of phosphorus and potassium fertilizer resulted in the second highest shoot and root yield, even at the 4.5 soil pH, with the best growth observed with the complete fertilizer (nitrogen, phosphorus, potassium) treatment. Seedhead production was increased at a 6.5 soil pH compared to a 4.5 soil pH.

Soil acidity affects annual bluegrass seed germination and establishment (Ferguson, 1936). It is thought that seeds are able to germinate, but are less able to establish a root system in the acidic soil. If all the plants in the stand are annual biotypes, then summer seasonal cycles of decline may slow or eventually stop the next generation from establishing. Annual bluegrass samples were collected in the United Kingdom from several golf course creeping bentgrass putting greens that had acidic (5.5 pH) sand based root zones. They observed that the greens contained no annual biotypes of annual bluegrass, with a perennial biotype being the main component of the annual bluegrass population (Hagley et al., 2002).

### Phosphorus and Potassium Effects on Annual Bluegrass Competitiveness

Working in the winter rainfall areas of South Africa in 1903, Dr. C. M. Murray observed that fertilizers containing phosphate increased annual bluegrass populations in lower cut turf. Soil phosphate levels ranging from 600 mg $kg^{-1}$ to well over 1000 mg $kg^{-1}$ increased annual bluegrass populations, while at 3 mg $kg^{-1}$, the stand was dominated by creeping bentgrass (Arthur, 2003). Applying phosphorus-containing fertilizers increases annual bluegrass growth relative to creeping bentgrass, even in unfavorable environments for annual bluegrass growth (Goss et al., 1977; Juska and Hanson, 1969; Lyons, 2004; Varco and Sartain, 1986).

Goss et al., (1977) showed that phosphorus applied at 195 kg $ha^{-1}$ $yr^{-1}$ resulted in increased annual bluegrass populations in a creeping bentgrass putting green. These results demonstrate the importance of sufficient phosphorus levels for optimal annual bluegrass growth. A fertilizer containing nitrogen and phosphorus applied at any rate can increase the annual bluegrass population when compared with areas where phosphorus was not applied. Even fertilizers containing sulfur and phosphorus increased annual bluegrass populations in creeping bentgrass greens, but a sulfur and nitrogen combination fertilizer decreased annual bluegrass populations (Goss et al., 1977).

Lawson (1999) conducted a study in the United Kingdom to determine the effects of phosphate (0, 4, 8, and 20 kg $ha^{-1}$) and potassium (0, 30, 50, and 126 kg $ha^{-1}$) fertilizers applied twice in the spring and fall to bentgrass growing on a sandy loam root zone. A higher application rate of phosphate increased annual bluegrass populations from 10% to 20% coverage and was further increased to 25% by potassium applications after eight years. Only the high phosphorus rates had a significant effect on annual bluegrass populations, indicating a threshold for phosphate levels (Lawson, 1999).

Phosphorus compounds commonly found in soils are mostly insoluble and unavailable for plant uptake. Even when soluble phosphorus forms are applied as fertilizers, subsequent soil reaction may transform the phosphorus into an insoluble form. Due to these reactions only about 10 to 15 percent of applied fertilizer phosphorus is thought to be readily available for plant uptake (Brady and Weil, 2008).

Phosphorus surface adsorption and precipitation reactions depend on soil pH. In acid soils, inorganic phosphorus precipitates as iron/aluminum-phosphorus secondary minerals and are adsorbed to surfaces of iron/aluminum oxide and clay minerals (Havlin et al., 2005). Due to the ubiquity of hydrogen ions in acid soils, the mineral surface has a net positive charge, even though both charges exist. The positive charge attracts hydrogen phosphate and phosphorus ions adsorb to the iron/aluminum oxide surface by interacting with hydroxide on the mineral surface. An acid soil fixes two times more phosphorus per unit surface area than neutral or calcareous soils and the phosphorus is held five times stronger to the surface area of the colloid. Phosphorus availability is maximized at a soil pH of 6.5 and decreases as pH goes down (Havlin et al., 2005). Sandy soils, such as golf greens built according to USGA recommendations, are commonly very low in iron or aluminum and do not form insoluble phosphorus complexes; under these conditions phosphorus is more available at a lower pH (McCarty, 2005).

### Sulfur

In the soil, elemental sulfur fertilizer applications are oxidized by *Thiobacillus* bacterial species into sulfuric acid. Once the sulfuric acid is formed it can easily disassociate, releasing the hydrogen ions that lower soil pH (McCarty et al., 2003). However, the addition of sulfate containing fertilizers contributes readily available sulfur as sulfate for plant uptake, but sulfate alone does not contribute to acidity except as hydrogen sulfate. Sulfate applied as ammonium sulfate can decrease soil acidity as a result of the oxidation of ammonium (Carrow et al., 2001).

Goss et al. (1977) observed the effects of sulfur (0, 56, or 154 kg ha$^{-1}$yr$^{-1}$) on a mixed stand putting green of 'Astoria' creeping bentgrass and annual bluegrass, located in Washington. The sulfur was applied two to three weeks apart in March to April at 56 kg S ha$^{-1}$ until the treatments received their respected rates. The 168.5 kg S ha$^{-1}$ yr$^{-1}$ rate significantly reduced annual bluegrass populations in a creeping bentgrass putting green and was increased when sulfur was applied at 56.2 kg ha$^{-1}$yr$^{-1}$. Sulfur applied at 168.5 kg S ha$^{-1}$ yr$^{-1}$ and the addition of phosphorus fertilizers (196 kg P ha$^{-1}$) increased annual bluegrass populations in the putting green and reduced the effect of sulfur for annual bluegrass control.

Varco and Sartain (1986) mixed different sulfur rates (0, 50, 100, or 150 kg S $ha^{-1}$) in the top 2.5 cm of the soil and observed emergence, establishment, and clipping yield of annual bluegrass seedlings. Sulfur incorporated into the surface soil decreased emergence, establishment, and clipping yield as the sulfur rate was increased. The highest sulfur rate (150 kg $ha^{-1}$) caused a decrease in the number of seedlings from 20 at 17 days to nine seedlings at 30 days. The increase in sulfur rate lowered soil pH in the top 2.5 cm, with the highest sulfur rate lowering soil pH from 5.6 to 4.4. As a result of these observations the authors determined that a soil pH near 5.0 would be adequate to reduce annual bluegrass seedling emergence, establishment, and clipping yield.

In an additional experiment, Varco and Sartain (1986) observed annual bluegrass seedling germination in varying acidic (4.3, 4.8, 5.3, and 7.0 pH) solutions over a 21 day period. A decrease in normal germination was observed as the pH decreased. The solution pH of 5.3 was determined to be the beginning of abnormal seedling germination. Abnormal growth was described as no signs of growth after emergence and the lack of a radicle emergence.

### Iron

One of the most widely used sources of iron for agriculture is ferrous sulfate (Havlin et al., 2005). Iron is an important micronutrient for turfgrass growth and maintenance. To encourage chlorophyll production turfgrass managers use iron applications to produce a darker green leaf blade (Carrow et al., 2001; Havlin et al., 2005). After application the grass is black at first, due to iron oxidation, but will turn green after 24 hours from application. In addition to increasing turf color, ferrous sulfate is also able to reduce soil pH. Ferrous sulfate reacts with oxygen and water to produce sulfuric acid and iron oxyhydroxide. The resulting sulfuric acid is the main source of soil acidity from ferrous sulfate (Brady and Weil, 2008).

Xu and Mancino (2001) observed the potential of iron for annual bluegrass suppression in creeping bentgrass by using 0, 2, 4, 6, and 8 mg $L^{-1}$ of iron on creeping bentgrass and annual and perennial annual bluegrass biotypes. Leaf color increased for all turf species as application rate of iron increased, but when iron was not applied light green color and intervenial chlorosis occurred, indicating a deficiency in iron. When no iron was applied, creeping bentgrass was observed to have a higher color rating than both of the annual bluegrass biotypes. As the iron rate increased the annual bluegrass perennial biotype produced a darker green color than the creeping bentgrass.

Xu and Mancino (2001) found that creeping bentgrass slightly decreased in dry shoot weight at the highest iron rate and still grew faster than both annual bluegrass biotypes. A peak growth rate for both annual bluegrass biotypes was observed for shoot and root dry weight at a low to medium rate producing no significant difference between the two biotypes. After the annual bluegrass biotypes reached a peak of iron tolerance, both biotypes declined as the iron rate increased. Root dry weight followed a similar trend as dry shoot weight of creeping bentgrass by resulting in more root growth than both the annual bluegrass biotypes. Creeping bentgrass root dry weight declined at the highest iron rate, while root weight for both annual bluegrass biotypes was significantly minimized. Observations indicated that increased iron treatments favor creeping bentgrass growth over annual bluegrass growth.

### Nitrogen

Ammonium is the preferred nitrogen source for plant uptake because it does not require extra energy for plants to assimilate. The frequent application of ammonium containing fertilizers can over time lower soil pH as the oxidation of ammonium occurs and is referred to as nitrification (McCarty et al., 2003). Nitrification is the transformation of ammonium to nitrate by soil microorganisms and requires adequate soil moisture, oxygen, and warm soil (>10$^{o}$C) temperatures to occur. In the soil the nitrosomonas bacteria react with ammonium nitrogen and oxygen and produce nitrite nitrogen, two hydrogen ions and water. The resulting release of the hydrogen ions decreases the soil pH over time (McCarty et al., 2003).

Lawson (1999) made the observation that ammonium sulfate (45 kg ha$^{-1}$, applied three times per year) reduced an initial annual bluegrass population coverage of 15% to less than 2% in a creeping bentgrass putting greens after eight years. The author attributed the decline in annual bluegrass populations to lowering soil pH and reducing available soil phosphorus. In an another study, ammonium sulfate (range of 69 to 402 kg ha$^{-1}$yr$^{-1}$) was applied every two weeks from April to October to a mixed annual bluegrass and creeping bentgrass putting green with a soil pH of 7. The ammonium sulfate caused a decrease in leaf phosphorus concentrations at 98 kg ha$^{-1}$ and was further reduced as the yearly application rate increased. The reduction of leaf tissue phosphorus concentrations lead to the decrease of annual bluegrass clipping yield (Schlossberg and Schmidt, 2007).

## Herbicides for Annual Bluegrass Control

Research trials have investigated the potential of the post-emergent herbicides bispyribac-sodium, ambicarbazone, ethofumesate, and methiozolin and a pre-emergent, dithiopyr for annual bluegrass control in creeping bentgrass putting greens. As of 2013 no registered herbicides are available that provide satisfactory pre or post annual bluegrass control without causing significant potential injury to creeping bentgrass managed under putting green conditions. These herbicides are also unable to transition the mixed stand to a monoculture without potentially losing putting green playability during the transition (Hart et al., 2004; McCullough et al., 2010; McCullough and Hart, 2010; Neylan et al., 1997; Teuton et al., 2007).

However, methiozolin has been observed to safely transition a mixed stand to a creeping bentgrass dominant surface without significant injury to creeping bentgrass (Askew and Koo, 2012). The current registered herbicide list includes two pre-emergent herbicides, oxadiazon and bensulide (Turgeon et al., 2009).

The application of bispyribac-sodium or ambicarbazone post emergent herbicides has been observed to be effective at controlling annual bluegrass in creeping bentgrass. However, after application of these herbicides discoloration of creeping bentgrass occurs and tolerance of creeping bentgrass to either herbicide depends on temperature, timing, and soil properties. These herbicides do not transition the population, but leave voids in the turf due to sudden death of annual bluegrass or creeping bentgrass. These herbicides are recommended for annual bluegrass control in creeping bentgrass tees and fairways and not recommended for putting green applications (McCullough and Hart, 2010; Teuton et al., 2007; McCullough et al., 2010). The combination application of ambicarbazone and paclobutrazol may increase creeping bentgrass tolerance to ambicarbazone and increase herbicide effectiveness for annual bluegrass control (Jeffries et al., 2010).

Ethofumesate was observed to control annual bluegrass (80-90%) in ‘Penncross’ creeping bentgrass putting greens for seven to eight months at several locations in North Carolina. However, injury was observed on the creeping bentgrass that lowered turf quality, but was considered acceptable for creeping bentgrass putting greens (Lewis and Dipaola, 1989). In contrast, ethofumesate was applied to creeping bentgrass putting greens that were infested with annual bluegrass in a temperate climate in Australia. The treatment of ethofumesate resulted in annual bluegrass reduction and an unacceptable thinning of creeping bentgrass and resulted in the early discontinuation of this herbicide trial. The authors did not recommend that ethofumesate be applied for annual bluegrass control in creeping bentgrass putting greens (Neylan et al., 1997).

Methiozolin was observed to reduce annual bluegrass from 58% to less than 5% with spring applications. A spring and fall application regime reduced annual bluegrass coverage to less than 15%. Quality was reduced during the transitioning of the turf to a monoculture, but may be considered acceptable by turf managers because of the reduction of annual bluegrass (Askew and Koo, 2012). The application of an experimental herbicide, methiozolin, resulted in less than 10% injury to creeping bentgrass, regardless of root zone soil type and application rate. Root injury was also observed with a reduction of root-length density from 9 to 25%. Methiozolin shows promise as an effective annual bluegrass control in creeping bentgrass putting greens despite minor and acceptable damage to creeping bentgrass (Brosnan et al., 2013).

Dithiopyr, a pre-emergent herbicide, caused a reduction in root mass no matter the application timing, eventually leading to a decrease in creeping bentgrass coverage. Recovery from application would take several months. Dithiopyr can potentially inhibit new root and shoot growth when applied in the fall and spring when turf is recovering from summer stress and actively growing. For these reasons dithiopyr is not recommended or labeled for use on creeping bentgrass putting greens (Hart et al., 2004).

Oxadiazon was observed to prevent the colonization of annual bluegrass in creeping bentgrass putting greens. Oxadiazon has been observed to cause leaf burn, but turf quality still was considered to be acceptable for a putting green and its application was recommended. The pre-emergent herbicide did not have an adverse effect on new root growth (Neylan et al., 1997).

A pre-emergent herbicide, bensulide, was observed to suppress annual bluegrass annual biotypes from germination, but had no control against established perennial biotypes. After repeated application for two years following recommend label rates, creeping bentgrass decline was seen in a small percentage of the putting green. The observed decline was attributed to the pre-emergent herbicide application (Callahan and McDonald, 1992). Bensulide reduces root mass and percent coverage of creeping bentgrass in the fall and spring. Bensulide application can be risky for creeping bentgrass putting greens. Spray overlap will often discolor and increase injury to the creeping bentgrass (Hart et al., 2004).

### Seaweed Extract

During the late-summer months, creeping bentgrass putting greens begin to decline in health, growth, and playability. This phenomenon is known as summer bentgrass decline. Turf health declines as a result of high air (35$^{o}$C) and soil temperatures (25$^{o}$C), which have negative physiological effects on the bentgrass (Xu and Huang, 2000). Damages due to these high air and soil temperatures are enhanced on annual bluegrass, which is not as tolerant to these extreme temperatures (Vargas, 2005). Numerous researchers have reported that turf quality, leaf chlorophyll, and protein content declines, while electrolyte leakage and lipid peroxidation increase during summer bentgrass decline (Huang et al., 1998; Liu et al., 2002; Xu and Huang, 2000). Root viability effects are an important factor influencing creeping bentgrass shoot growth under high temperature stress because roots are the primary site for the biosynthesis of cytokinins (Huang et al., 1998).

Seaweed extract (SWE) has been reported to contain zeatin riboside, a sugar derivative form of cytokinin (Zhang and Ervin, 2004). Foliar exogenous applications of SWE have been shown to result in a reduction in summer bentgrass decline. Similar results were reported with the application of zeatin riboside injected into the root zone, increasing endogenous cytokinin content in both shoots and roots, alleviating heat stress injury (Liu et al., 2002). An additional benefit of SWE is the regulation of the plant cell membrane; mainly phosphate lipids and free sterols, influencing fluidity and permeability of the membrane structure increasing drought resistance (Yan et al., 1997). The beneficial influences of SWE on turf abiotic stress tolerance are associated with hormonal components, not the minerals found in the extract (Zhang and Ervin, 2004).

SWE has been observed to increase the tolerance of non-target or desired turf species to post and pre-emergent herbicides. Zhang et al. (2001) observed the influence of a combination of Trimec® (bentgrass formula, 2, 4-D + Mecoprop + Dicamba) and SWE for white clover (*Trifolium repens* L.) control and photochemical activity in creeping bentgrass. Compared to Trimec-alone, the combination decreased injury to the creeping bentgrass. In another study, Schmidt and Luo (1993) observed the influence of pendimethalin (3.3 and 6.6 kg ai ha$^{-1}$) applied six weeks after planting Kentucky bluegrass (*Poa pratensis* L.) seeds treated with SWE (13 g of product per kg of seed) prior to seeding. The low herbicide dosage inhibited shoot growth, while the high dosage decreased root mass. The pendimethalin and SWE combination increased root development compared to pendimethalin applied alone, suggesting that SWE may reduce potential non-target negative effects of a pre-emergent herbicide.

## Paclobutrazol

Gibberellic acid is a plant hormone involved in stimulating shoot elongation, delaying leaf senescence, breaking seed dormancy, promoting seed germination and inducing flowering. When gibberellin production is inhibited and cell elongation is reduced, the internodes become shortened and the plant is smaller (McCarty, 2005). Paclobutrazol (Pac) is a class B plant growth regulator (PGR) that inhibits the synthesis of gibberellic acid. Pac is more effective at gibberellic acid inhibition compared to other plant growth regulators in its class (McCarty, 2005).

Turf managers use Pac to reduce vertical growth of creeping bentgrass in putting greens. The reduction in vertical growth reduces mowing frequency, encourages thicker turf stands, and increases putting speed (McCarty, 2005). Pac is metabolized slower than class A PGRs. Suppression duration can last from two to eight weeks, depending on environmental conditions (McCarty, 2005).

Introduced in the 1980's, Pac has been shown to suppress annual bluegrass growth more than creeping bentgrass, favoring creeping bentgrass dominance. The PGR application is able to reduce the growth of both annual and perennial species in a mixed stand and maintain the creeping bentgrass dominance (Woosley et al., 2003; Baldwin et al., 2010; Johnson and Murphy, 1996). Compared with herbicides that have been studied for annual bluegrass control, Pac has been found to be the most effective at transitioning a mixed culture to a monoculture without significant damage to creeping bentgrass (Woosley et al., 2003). Treated creeping bentgrass is able to metabolize the growth regulator faster than the annual bluegrass, allowing the bentgrass suppression period to be shorter. A shorter suppression period allows the bentgrass to take over the slower growing annual bluegrass and eventually outgrow the weed (McCarty, 2005).

The standard recommendation for using Pac (22.9% ai,Trimmit 2SC) to control annual bluegrass populations in creeping bentgrass putting greens is to apply 0.04 to 0.27 kg ai ha$^{-1}$, every fourteen days, spring through the fall. Another recommendation that is also effective at annual bluegrass control is applying Pac at a rate of 0.40 kg ai ha$^{-1}$ and applying two to three times in fall and in the spring at four week intervals. It is recommended that Pac should be applied during periods of active creeping bentgrass root growth (McCarty, 2005).

High spring and fall application rates can cause damage or be phytotoxic to the creeping bentgrass and recovery from injury can take three to four weeks. Late-fall applications can turn the turf a tan color that may last through the winter, when temperatures reach 0°C during the night (Woosley et al., 2003; Baldwin et al., 2010; Johnson and Murphy, 1996). In addition to possible phytotoxicity to the creeping bentgrass, the sudden prevention or failure to continually apply Pac can result in a rebound effect. A rebound effect is when the turf has a surge of vertical growth in a short amount of time as a result of no Pac application. The cessation of Pac after a regular treatment program can increase annual bluegrass populations that were once suppressed by the frequent, previous Pac applications (McCarty, 2005).

### Silvery Thread Moss Description

Out of the 9,500 moss variations that occur, silvery thread moss is the most common moss found on creeping bentgrass putting greens. *Bryum argenteum* Hedw., commonly called silvery thread moss, is a primitive, nonvascular, non-parasitic plant that does not have roots. The common name comes from the silvery glare of moss patches in the sunlight. The moss is able to survive in shaded, poorly drained sites or in sunny, dry sites. Silvery thread moss photosynthesizes and fixes nitrogen, allowing it to survive in these varying environmental conditions (McCarty, 2005).

Silvery thread moss colonizes creeping bentgrass putting greens when creeping bentgrass health begins to decline. Establishment typically occurs in the summer following periods of rainy, overcast, warm days (Turgeon et al., 2009). The moss is mainly found on putting green ridges or mounds where the grass becomes thinned from scalping or drought. Summer establishment is ideal for moss population growth due to a slow growing, struggling bentgrass stressed by summer heat (Turgeon et al., 2009). Moss is distributed to these areas by spores and the physical movement of the moss through golf spikes, mowers, or other turf equipment (McCarty, 2005).

## Moss Control Methods

Few herbicides have been registered for use on bentgrass putting greens for silvery thread moss control. The effectiveness of these registered herbicides varies due to environmental conditions and the season of application. All registered herbicides require repeated applications for continuous colony reduction and prevention of new colony establishment. Carfentrazone, the only currently registered herbicide for moss control, and two fungicides, mancozeb plus copper hydroxide and chlorothalonil, have been occasionally reported to provide effective control.

Carfentrazone is effective at reducing moss populations in creeping bentgrass putting greens. The herbicide does not damage creeping bentgrass, allows a safe transition to a creeping bentgrass monoculture, and is effective at injuring moss foliage. Because the herbicide requires contact with moss foliage, spray applications can miss the moss tissue and undamaged tissue can regrow. For this reason multiple applications are required for population reduction and control (Borst et al., 2010; Kennelly et al., 2010).

Carfentrazone plus cultural practices increase herbicide efficacy. These cultural practices include nitrogen fertilizer applications and applying sand topdressing. Foliar ammonium sulfate applied weekly at 4.9 to 6.1 kg ha$^{-1}$ promotes a more competitive bentgrass, helps desiccate moss, and also acidifies the soil to discourage moss (Turgeon et al., 2009). Carfentrazone (0.1 kg ai ha$^{-1}$) plus nitrogen (12.2 kg ha$^{-1}$) reduced silvery thread moss populations by 39% (Borst et al., 2010). Carfentrazone plus topdressing and carfentrazone plus nitrogen and topdressing reduced populations by 73 and 66%, respectively. Topdressing followed by brushing the sand into the turf canopy increased carfentrazone efficacy by breaking up the plants and allowing more surface area to come into contact with the herbicide.

Mancozeb plus copper hydroxide is a fungicide and bactericide that has been observed to be effective at controlling silvery thread moss in creeping bentgrass putting greens. Borst et al. (2010) observed effects of the bi-weekly application of mancozeb plus copper hydroxide (1.8 kg $ha^{-1}$) in the early-summer on silvery thread moss colonies in creeping bentgrass putting greens for 10 weeks. Three weeks after the initial application, 11% control was observed, increasing to 15% at five weeks, with control declining after five weeks. The authors attribute this low control to early-summer application and recommend that fall applications are most effective because the creeping bentgrass is more competitive with the moss colonies during this time. The herbicide has potential to damage creeping bentgrass with multiple, continuous applications at label rates (Borst et al., 2010).

Registered for use on golf courses since the 1960's, chlorothalonil, a multi-site contact fungicide (with and without zinc), has been observed to be as effective as carfentrazone in controlling silvery thread moss (Kennelly et al., 2010; Burnell et al., 2004). Chlorothalonil is mainly used to control dollar spot and requires frequent application at 7 to 14 day intervals for effective control. Chlorothalonil (8.2 and 12.8 kg ai $ha^{-1}$) applied bi-weekly, April through October, has been observed to reduce silvery thread moss colony diameters after repeated applications. A low rate of chlorothalonil (8.2 kg ai $ha^{-1}$) + mancozeb (9.9 kg ai $ha^{-1}$) + thiram (11.6 kg ai $ha^{-1}$) reduced moss populations, colony diameters, and was as effective as a high rate of chlorothalonil (Kennelly et al., 2010). For chlorothalonil to be an effective herbicide for silvery thread moss control the fungicide requires drying on the leaf surface as irrigation after application reduces effectiveness. The addition of zinc to the formulation increased its moss control effectiveness (Burnell et al., 2004).

Heavy metals, such as zinc or iron, displace the central magnesium atom in chlorophyll causing a decline in silvery thread moss (Turgeon et al., 2009). Burnell et al. (2004) found that iron treatments reduced populations as opposed to treatments that did not receive iron. Ferrous sulfate (40 kg ha$^{-1}$) + ammonium sulfate (30 kg N ha$^{-1}$) applied at four week intervals reduced populations by 67% after two weeks and 87% after six weeks. By ten weeks, the population reduction had fallen to 67% due to ferrous sulfate and ammonium sulfate immediate release of elements after application.

Previous research suggests that frequent, high iron rates can decrease annual bluegrass root and shoot growth while creeping bentgrass growth is increased (Xu and Mancino, 2001). However, bi-weekly, high ferrous sulfate rates have not been researched in a field study with the objective of transitioning a mixed stand of creeping bentgrass and annual bluegrass to a creeping bentgrass monoculture. In addition, SWE is known to safen herbicide applications for non-target species, but it is unknown if it suppresses annual bluegrass or if it can safen frequent, high ferrous sulfate rates. Pac has been observed to transition a creeping bentgrass putting green cohabitated with annual bluegrass, but it is unknown if a combination with ferrous sulfate will safen and quicken the transition (Woosley et al., 2003; Baldwin et al., 2010; Johnson and Murphy, 1996). The objective of the study herein is to determine the effects of repeated high rates of ferrous sulfate on transitioning an annual bluegrass-infested creeping bentgrass putting green to a monoculture. The secondary objectives are to determine if SWE with ferrous sulfate safens the transition to a creeping bentgrass monoculture. Our last objective is to determine if a high Pac rate with or without ferrous sulfate is a safe combination for annual bluegrass suppression in creeping bentgrass putting greens in a northern transition zone climate.

## Materials and Methods

The trial was arranged as a randomized complete block split-plot design with four main plots consisting of three ferrous sulfate rates and three subplots consisting of no PGR, SWE, or Pac. The study was repeated in time over two years on the same area of a 25-year-old ‘Penneagle’ creeping bentgrass putting green at the Virginia Tech Turfgrass Research Center in Blacksburg, VA. At trial initiation the green was infested with over 45% naturally-occurring annual bluegrass and less than 5% silvery thread moss. The green was built with a 20 cm sandy loam root zone over 5 cm of pea gravel. At trial initiation soil pH was 6.4, and phosphorus and potassium soil levels were at 8 and 14 mg kg$^{-1}$, respectively. Soil iron levels were 82 and 93 mg kg$^{-1}$ at 2.5 and 10.2 cm depths, respectively. Based on recommend soil nutrient concentrations for turfgrass, phosphorus and potassium levels at the trial site were low, while the iron was high (McCarty, 2005). Irrigation water (pH 7.6, VWR Scientific Products SR 601C, Radnor, PA) was applied as needed to prevent visual signs of wilt. The green was mowed five times per week at 3.2 mm. Chlorothalonil (Daconil Zn®, Syngenta Professional Products, Greensboro, NC) was used at the recommended label rates and rates varied according to disease pressure to maintain acceptable levels of dollar spot and *Rhizoctonia* brown patch control. The trial site was aerified in the spring (Mar. 21, 2011 and Mar. 14, 2012) and fall (Sept. 9, 2011 and Oct. 10, 2012), totaling 15% surface area removal per year, followed by pure sand topdressing that was brushed in.

The main plots were 2.7 by 1.8 m and treated with ferrous sulfate (19% iron and 11% sulfur, Hi-Yield®, Bonham, TX) at 0.0, 12.2, 24.4, and 48.8 kg ha$^{-1}$. Subplots were 0.9 by 1.8 m and treated with no PGR, SWE (0-3-2, Pana Sea® plus Liquefied Sea Plant Extract, Ann Arbor, MI) at 12.8 L ha$^{-1}$, or Pac (22.9% ai, Trimmit 2SC, Syngenta, Greensboro, NC) at 0.37 kg ai ha$^{-1}$ (March 1 to June 1 and September 1 to October 31) and 0.18 kg ai ha$^{-1}$(June 1 to August 31). All products were dissolved or suspended in water (7.6 pH) and applied by a $CO_2$-powered (276 kPa at 374 L ha$^{-1}$)

walk behind sprayer with XR TeeJet 8003VS nozzles. Ammonium sulfate (21% nitrogen and 22% sulfur, Hi-Yield®, Bonham, TX) was sprayed uniformly over the study area every two weeks from March through November for a total of 18 treatments at 4.8 kg N ha$^{-1}$ each. Ferrous sulfate, SWE and Pac were applied bi-weekly starting on March 2, 2011 and March 4, 2012; final annual applications were October 24, 2011 and October 26, 2012.

The Pac rates for this experiment are not based on product label recommendations (Anonymous, 2012). When applying Pac continuously throughout the growing season, it is typical to apply on a routine schedule, but reduce the rate, often by half, during summer stress. In this experiment, our normal rate was selected to represent the most aggressive rates used by superintendents in the northern or mountainous regions of Virginia (Hagood and Herbert, 2012). This rate is 1.5 times more than the maximum label rate. This aggressive rate was applied 11 times and half the rate an additional 7 times for each growing season. Thus, the Pac use in this study equates to 4.5 times the labeled annual allowable use. The use of such excessive rates was not meant to represent current management practices, but rather to create a worse-case scenario whereby creeping bentgrass tolerance to both aggressive iron and Pac concentrations could be assessed.

Annual bluegrass infestation percentage was estimated when sufficient seedheads were observed in turf plots from late-winter to mid-spring, depending on weather conditions. Percent infestation was estimated using a grid measuring 0.9 m x 1.8 m with 512 squares each square being 25 cm$^2$. The grid was placed on each sub-plot and the presence or absence of annual bluegrass in each square was scored. Annual bluegrass infestation was assessed instead of annual bluegrass cover because accurate assessment of cover was hindered by ferrous sulfate, which obscured annual bluegrass and creeping bentgrass differentiation. The probability of correctly identifying at least one annual bluegrass plant in each square was better than identifying every plant. The presumption was that a misrepresentation of annual bluegrass cover was less desirable than an accurate representation of infestation.

Color and quality was rated one week after each biweekly treatment during the growing season between March and November. Color ratings were based on a 1-9 scale with 1 = brown turf, 6 = minimally-acceptable green turf, and 9 = dark green turf. Quality ratings were based on a 1-9 scale with 1 = dead, poor turf, 6 = minimally-acceptable turf, and 9 = healthy, high quality turf.

Soil samples were collected to determine the pH, phosphorus, potassium, and iron levels at trial initiation each March and conclusion each December. Samples were taken at 2.5 and 10.2 cm soil depths. Samples were processed at the Virginia Tech Soil Testing Laboratory located in Blacksburg, VA using Mehlich buffer solution and following Mehlich I extraction procedures (Maguire and Heckendorn, 2011).

A count of silvery thread moss colonies (measuring between 5 to 30 mm in diameter) was taken at the end of each growing season, in December. An outbreak of dollar spot occurred in September 2011 and disease infection centers were counted as ancillary data for that year.

Data were subjected to a combined analysis of variance (ANOVA) using the general linear models procedure in SAS version 9.1 (SAS Institute, Cary, NC) with sums of squares partitioned to reflect the split-plot treatment design and the two years as a random factor. Mean squares were tested as appropriate for the split-plot design and random years (McIntosh, 1983). Interactions and main effects were separated with Fisher's protected LSD test ($\alpha = 0.05$) or described with linear or polynomial regressions where appropriate. Assessments that were repeated in time, such as color and quality ratings were averaged to avoid variance structure. It was noted, however, that color and quality were generally consistent from one bi-weekly rating to the next, but tended to differ between seasons. Therefore, color and quality ratings were averaged by season prior to ANOVA. In the case of annual bluegrass infestation and moss colony counts, data were analyzed separately by each assessment date.

## Results

### Annual Bluegrass Infestation Percentage

The ANOVA test for annual bluegrass infestation percentage as affected by ferrous sulfate rates, PGR treatments (control, SWE or Pac) or their combination over time on a creeping bentgrass putting green are presented in Table 1. A significant interaction between ferrous sulfate and PGR was observed and the data are presented in Figure 1 pooled over years due to lack of trial interaction.

The annual bluegrass percent infestation was estimated to be approximately 45% at trial initiation. With each 10 kg ha$^{-1}$ increase in ferrous sulfate rate, annual bluegrass infestation decreased 2.8 and 2.9% when no PGR and SWE were applied, respectively (Figure 1). The aggressive Pac program effectively controlled annual bluegrass regardless of ferrous sulfate rate (Figure 1). It should be noted that a 15% decline in annual bluegrass occurred in plots that did not receive PGR or ferrous sulfate, possibly due to creeping bentgrass competition being favored by biweekly application of ammonium sulfate and exclusion of phosphorus and potassium fertilizers.

### Color

The ANOVA test for turf color rating is presented in Table 2. The year by ferrous sulfate rate interaction was significant for summer and fall and the ferrous sulfate main effect was significant during all three seasons. In comparing data between the two years, the cause of this interaction was not apparent as it appeared the same trends occurred each year. Therefore, color data from each season were pooled over years to display the ferrous sulfate main effect in Figure 2. With each 10 kg $ha^{-1}$ increase in ferrous sulfate, turf color increased by 0.4, 0.2, and 0.4 in spring, summer, and fall, respectively (Figure 2). The ANOVA test presented in Table 2 also indicated a significant interaction for the PGR treatments for summer and fall (data not shown). In summer and fall, Pac treatments improved turf color to 7.7 and 7.5, respectively; ratings that were better than the control or SWE.

### Quality

The ANOVA test for turf quality ratings as affected by ferrous sulfate rates, PGR treatments (control, SWE or Pac) or their combination over time on a creeping bentgrass putting green are presented in Table 2. The interaction for ferrous sulfate was significant and is presented in Figure 3.

As ferrous sulfate increased from 0 to 24.4 kg $ha^{-1}$, turf quality increased, regardless of season (Figure 3). Increasing ferrous sulfate rate above 24.4 kg $ha^{-1}$ either did not improve turf quality further or slightly decreased quality (Figure 3). The interaction of year by PGR was significant during the spring and summer (Table 2). Typically, Pac treated plots had higher quality than SWE or control plots and SWE seldom influenced turf quality compared to the control (data not shown). The year interaction likely occurred because Pac-treated plots were equivalent to control plots in spring 2011 and summer 2012 with quality of 6.1 and 7.1, respectively. In contrast, Pac-treated plots had superior quality compared to control plots in summer 2011 and spring 2012 with ratings of 6.6 and 6.7, respectively (data not shown).

### Soil pH and Nutrient Availability

The ANOVA test for soil pH and nutrient availability as affected by ferrous sulfate rates, PGR treatments (control, SWE or Pac) or their combination over time on a creeping bentgrass putting green are presented in Table 3. No significant interactions were noted for any edaphic variables (Table 3). The main effect of ferrous sulfate was significant for pH assessed at a 2.5-cm depth and iron assessed at a 10.2-cm depth (Table 3). The main effect of PGR was significant for pH, phosphorous, potassium, and iron each assessed at a 2.5-cm depth (Table 3).

For every 10 kg ha$^{-1}$ increase in ferrous sulfate, pH decreased 0.04 units at a 2.5 cm soil depth (Figure 4). Ferrous sulfate did not influence pH at 10.2 cm (Table 3). Increases in ferrous sulfate rate also decreased iron levels at a 10.2 cm depth (Figure 4) but did not influence iron levels at a 2.5 cm depth (Table 3).

At the 2.5 cm soil sample depth pH for SWE and Pac plots were not significantly different from each other, but were slightly higher than the control plots (Table 4). The SWE plots had slightly higher soil phosphorus levels than plots treated with Pac. The soil potassium levels in plots treated with SWE were higher than the control. The control plots had higher iron soil levels compared to Pac, which had the lowest concentration.

### Silvery Thread Moss

Analysis of variance indicated significant interactions for year by ferrous sulfate and ferrous sulfate by PGR for silvery thread moss colonies (Table 5) as well as main effects for ferrous sulfate and PGR.

Generally, addition of ferrous sulfate tended to decrease moss colony counts regardless of PGR (Figure 5). Addition of Pac, however, dramatically increased moss colony counts when ferrous sulfate was not applied but increasing ferrous sulfate rate reduced moss colonies, such that equivalent colony counts were observed in all PGR treatments at the highest ferrous sulfate rate (Figure 5). In the presence of the aggressive Pac program, each 10 kg ha$^{-1}$ increase in ferrous sulfate eliminated 8 moss colonies m$^{-2}$ while the same increase in ferrous sulfate eliminated 1 and 2 moss colonies m$^{-2}$ in the control and SWE plots, respectively (Figure 5).

### Dollar Spot

After one year of treatment applications (September 2011), a dollar spot outbreak was observed as a result of a failed fungicide application. This disease outbreak presented an opportunity to determine if treatments influenced disease incidence. Analysis of variance indicated that both ferrous sulfate rates and PGR main effects were significant but did not interact for counts of dollar spot infection centers (Table 6). When averaged over PGR effects, each 10 kg ha$^{-1}$ increase in ferrous sulfate rate eliminated approximately 9 dollar spot infection centers (Figure 6). When averaged over ferrous sulfate rates, the control and the SWE had higher dollar spot counts compared to Pac (Table 7).

## Discussion

Annual bluegrass infestation was estimated to be approximately 45% at trial initiation. As ferrous sulfate rate increased, a decrease in annual bluegrass infestation was observed, regardless of PGR treatment. Addition of SWE with or without ferrous sulfate did not affect annual bluegrass percent infestation or turf color and quality during the two year trial. Ferrous sulfate in combination with Pac had little effect on annual bluegrass infestation, due to the strong annual bluegrass control effects of Pac alone.

Ammonium sulfate and exclusion of phosphorus and potassium in the general greens maintenance program that was initiated with these trials may explain the annual bluegrass coverage reduction observed in the control over time (Figure 1). Lawson (1999) observed similar results of annual bluegrass reduction with ammonium sulfate fertilizer applications.

Over time, ammonium in the fertilizers oxidizes and acidifies soil, decreasing the availability of soil phosphorus for plants, disfavoring annual bluegrass (McCarty et al., 2003). Numerous researchers have come to similar conclusions after conducting putting green field studies and greenhouse trials that by lowering soil pH and reducing phosphorus availability, annual bluegrass populations declined in mixed turf stands. Even at a soil pH (below 5.5) that is detrimental to annual bluegrass stands, the addition of phosphate and/or potassium containing fertilizers can increase annual bluegrass populations (Goss et al., 1977; Juska and Hanson, 1969; Varco and Sartain, 1986; Lawson, 1999).

The annual bluegrass reduction caused by ferrous sulfate in creeping bentgrass may be explained by the conclusions made by Xu and Mancino (2001). These authors observed a decrease in annual bluegrass root and shoot growth at frequent iron applications of 7.6 kg iron $ha^{-1}$, while creeping bentgrass slightly declined at 10.1 kg Fe $ha^{-1}$. These data support our findings that frequent, high rates of ferrous sulfate used in this trial (2.3 to 9.2 kg Fe $ha^{-1}$) favored creeping bentgrass growth over the colonizing annual bluegrass. The high ferrous sulfate rate was more detrimental to annual bluegrass health than the medium rate; therefore, the high ferrous sulfate reduced annual bluegrass quicker than the medium rate.

In previous studies, Pac has not controlled annual bluegrass but does reduce annual bluegrass cover (Woosley et al., 2003; Baldwin et al., 2010; Johnson and Murphy, 1996). For this trial, the higher-than-labeled Pac rate quickly reduced annual bluegrass infestation but even in this aggressive Pac program, approximately 5% annual bluegrass infestation remained, regardless of ferrous sulfate rate (Figure 1). The actual annual bluegrass coverage could not be assessed due to confounding effects of ferrous sulfate but it was estimated to be less than 1% in Pac plots at the conclusion of this study. The standard recommendation for using Pac (22.9% ai, Trimmit

2SC) to control annual bluegrass populations in creeping bentgrass putting greens are biweekly applications (0.04 to 0.27 kg ai ha$^{-1}$), spring through fall. These Pac rates are recommended to prevent possible phytotoxicity to the creeping bentgrass that can occur if higher rates are applied (McCarty, 2005). The Pac rate used in our trial was 0.37 kg ai ha$^{-1}$ every two weeks in the spring and fall and 0.18 kg ai ha$^{-1}$ in the summer. The higher than recommended Pac rate used in this trial explains the rapid reduction in annual bluegrass percent infestation, while retaining acceptable turf quality.

During the growing season, plots treated with Pac had darker green color compared to the lighter green color for the control. Reduced cell elongation creates a leaf blade that has higher chlorophyll content, causing the grass to be darker green in color (McCarty, 2005). However, on a few rating dates in late-spring and late-fall the Pac reduced turf color ratings, indicating phytotoxicity from the Pac application. The phytotoxicity observed during these seasons has also been observed in previous research trials (Woosley et al., 2003; Baldwin et al., 2010; Johnson and Murphy, 1996). Because of the darkening effect caused by the ferrous sulfate application, Pac phytotoxic symptoms were effectively masked during these periods.

The high ferrous sulfate rate turned the creeping bentgrass to a dark green to black color, which lasted more than two weeks. The turf darkening to an almost black color as a result of the ferrous sulfate may be aesthetically unacceptable for turf managers of creeping bentgrass putting greens (Figure 7). However, the low and medium ferrous sulfate rates turned the turf plots a consistent dark green color throughout the seasons and may provide a darker green color that is more acceptable for creeping bentgrass putting greens (Figure 8).

The low ferrous sulfate rate increased turf quality compared to the 0 ferrous sulfate and slowly transitioned the mixed stand to a creeping bentgrass monoculture without reducing turf quality.

The medium and high ferrous sulfate rates consistently provided the highest quality turf for the trial. The medium ferrous sulfate rate increased turf quality during both summers of treatment and safely transitioned the mixed stand to a monoculture (Figure 8). However, the highest ferrous sulfate rate had consistently high quality during the trial, except during the late-summer when quality declined as a result of rapid annual bluegrass decline. During the mid- to late-summer months creeping bentgrass quality was reduced due to the highest ferrous sulfate rate to unacceptable levels during extreme heat of 35$^{o}$C or above. A gradual thinning of turf was seen until the spots became bare, about the size of a quarter. Only a few bare spots were observed in each plot treated with the high ferrous sulfate rate (Figure 9). After turf thinning during the summer of 2011, the voids were completely filled in with turf the following spring when applications began again in March. During the summer of 2012, the turf thinning was observed, but not to the extent observed the previous summer. These bare spots or turf thinning are thought to be annual bluegrass populations collapsing as a result of the ferrous sulfate application and summer stress. No annual bluegrass plants were observed to germinate into these thinned out and bare areas.

The observed soil pH drop at 2.5 cm depth resulted from the ferrous sulfate and ammonium sulfate applications. The uniform application of ammonium sulfate across all plots lowered soil pH and most likely was the main cause for slight uniform reductions in soil pH. The ferrous sulfate contributes a smaller amount of hydrogen ions than ammonium sulfate and helps explain why only the highest ferrous sulfate rate was significantly different from the lower ferrous sulfate rates in terms of soil pH (Havlin et al., 2005).

After two years of the bi-weekly treatment of ammonium sulfate and ferrous sulfate fertilizers a pH change was observed. The decline in pH may have been slowed by the basic pH of the irrigation water and the slow movement of ammonium and sulfate into the root zone. The irrigation water has a pH of 7.6 and a calcium level of 12 mg $kg^{-1}$, which most likely counteracted the acidifying effects of the ammonium sulfate and ferrous sulfate. The bicarbonate and carbonate in the irrigation water reacts readily with calcium in the irrigation water and soil to form calcium carbonate, which has a low solubility. This reaction decreases the effectiveness of ammonium and sulfates to reduce soil pH levels since calcium carbonate is insoluble and is favored in the soil (Carrow and Duncan, 1998). In addition, when applied to the soil surface the downward movement of the oxidized sulfur and ammonium into the root zone is slow, with a dramatic pH decrease in the thatch layer and a minimal effect on the root zone (McCarty et al., 2003).

At the 10.2 cm soil depth a significant difference between ferrous sulfate rates and soil iron concentration was observed. Higher soil iron concentrations were observed in the low and no ferrous sulfate plots, while the medium and high ferrous sulfate rates were observed to be the lowest. The reason for this observation is unknown and is most likely of little biological significance in terms of the plant responses observed in this trial. In addition to this iron concentration observation, a significant difference in soil iron concentration was observed in the control and Pac plots at 2.5 cm soil depth. A possible explanation for this observation is that the control had a lower, acidic soil pH and at this pH, more iron is readily available in soil solution (Carrow et al., 2001).

A significant difference was observed for the phosphorus and potassium soil concentrations at 2.5 cm depth between the SWE plots and the control and the Pac plots. The higher phosphorus and potassium soil concentrations can most likely be attributed to the SWE (0-3-2) that contains a small amount of these elements. The bi-weekly treatments from March through October, most likely ensured that small increases in soil available phosphorus and potassium would occur. However, once again, these differences were slight and most likely contributed very little to plant responses of interest in this trial.

When samples were taken from the thatch layer of plots treated with the highest ferrous sulfate rate, a thin (1 to 2 mm) black layer was observed (Figure 10). The low, medium, and no ferrous sulfate rates did not have a visible black layer in the thatch (Figure 10). The frequent application of sulfur and iron containing fertilizers has the potential to create a black layer. The layer is formed under anaerobic soil conditions when sulfate is used by soil microorganisms rather than oxygen because a gaseous hydrogen sulfide is created. A black colored layer develops from the concentration of iron sulfide and other ions of sulfide prevent the movement of water downward in the soil profile. Hydrogen sulfide is toxic to plants and causes thinning of turf due to the unstable gas of hydrogen sulfide (McCarty, 2005; Vargas, 2005). This thin black layer did not result in a decline in turf health other than minor summer thinning and has not functioned as an impediment of water infiltration into the soil (Reams, personal observation). The black layer build up can be controlled by frequent and consistent aerification practices in combination with sand topdressing each year, removing a minimum of 15% surface area (McCarty, 2005).

The observation of a black layer probably caused by the high ferrous sulfate rate was not to be expected since ferrous iron is water soluble and would be expected to leach through the soil profile (Havlin et al., 2005). Ferrous sulfate may be accumulating on the soil surface rather than leaching into the soil column and is diluted or carried away in drainage water. A trade magazine article has reported that frequent applications of iron containing products to putting greens built to USGA rootzone specifications has resulted in an accumulation of iron at the gravel and sand root zone interface. This accumulation has resulted in reduced drainage through the soil profile and is under further investigation (Obear, 2013). However, under our study conditions the sandy loam root zone may have slowed the percolation of iron through the root zone (Brady and Weil, 2008). The slow percolation of water and soluble ferrous iron may have caused the iron to accumulate in the thatch layer and soil interface.

Burnell et al. (2004) observed that iron applications reduced silvery thread moss populations in creeping bentgrass. Our data support Burnell's findings. For turf managers, the medium and high ferrous sulfate rates satisfactorily controlled silvery thread moss populations. However, the aggressive application rate of Pac increased silvery thread moss populations, even at the medium ferrous sulfate rate. Silvery thread moss was able to compete with the creeping bentgrass because of the high Pac rate and application frequency that suppressed creeping bentgrass growth. The high ferrous sulfate rate and Pac combination was able to control moss colonies as well as the medium and high ferrous sulfate applications.

During the fall of 2011, thiophanate-methyl (Cleary's 3336, Dayton, NJ) was applied as a preventive fungicide against *S. homoeocarpa* infection. A few days after application, *S. homoeocarpa* mycelia and infection centers were observed throughout the trial area. *S. homoeocarpa* has been determined to be resistant to this fungicide class and is why the fungicide was ineffective at infection prevention (McCall, personal communication). A visual comparison between the control and high ferrous sulfate plots indicated that ferrous sulfate may be reducing *S. homoeocarpa* infection. As a result of this observation, a count of the individual infection centers per plot was conducted.

Pac had fewer dollar spot infection centers compared to the control and SWE plots. This observation is in accordance with previous dollar spot control studies with creeping bentgrass (Bishop et al., 2008; Fidanza et al., 2006; Golembiewski et al., 1995; Miller et al., 2002; Ok et al., 2011). Even though dollar spot reduction was observed due to Pac applications, this treatment alone did not control dollar spot sufficiently to meet the standards for turf managers of less than 3% coverage of infection centers on creeping bentgrass putting greens (McCall, personal communication).

The medium and high ferrous sulfate rates had significantly lower dollar spot infection centers than the low and no ferrous sulfate, which were not significantly different from each other. These high ferrous sulfate rates suppressed and controlled dollar spot infection in the early-fall when disease pressure was high. Little to no research considering ferrous sulfate as a fungistat for dollar spot control has been conducted and warrants further investigation.

## Conclusion

Bi-weekly applications of ferrous sulfate can produce desirable results for annual bluegrass, silvery thread moss, and dollar spot control, but may also have a negative effect on turf quality. The annual bluegrass coverage decrease in the 0 ferrous sulfate indicates that ammonium sulfate and refraining from the application of phosphorus and potassium fertilizers can slowly reduce annual bluegrass coverage. The low ferrous sulfate rate increases turf quality compared to 0 ferrous sulfate and slowly suppresses annual bluegrass populations, but is not as effective in annual bluegrass or silvery thread moss control as the higher ferrous sulfate rates. The medium ferrous sulfate rate increased turf quality (even during summer stress), almost transitioned a mixed stand of annual bluegrass and creeping bentgrass to a bentgrass monoculture, and suppressed silvery thread moss colonies. The high ferrous sulfate rate is effective at lowering annual bluegrass populations, silvery thread moss, and controlling dollar spot, but caused a dark green to black color that may be aesthetically unacceptable to golf course superintendents. In addition, during the late-summer months the highest ferrous sulfate rate can cause annual bluegrass populations to quickly thin, causing voids in turf that can take weeks to adequately recover density.

The bi-weekly, aggressive Pac rate (0.37 L ai $ha^{-1}$ spring and fall; 0.18 L ai $ha^{-1}$ summer) can quickly and safely transition a 45% annual bluegrass infestation in creeping bentgrass putting greens to less than 10% infestation in upper transition zone climates. However, this aggressive rate in the spring and fall can cause phytotoxicity to the creeping bentgrass, lowering turf color. Creeping bentgrass discoloration caused by Pac can be masked by all three ferrous sulfate rates tested in this study. Because of the aggressive Pac application rate and frequency, creeping bentgrass growth was slowed enough to allow silvery thread moss colonies to thrive. To counteract the Pac effects, the high ferrous sulfate rate was needed to effectively suppress moss colonies. This observation warrants further investigation into the effect of the different plant growth regulators, DMI fungicides, and varying rates of each on silvery thread moss colonies.

After two years of continuous application, the ammonium sulfate was effective at lowering soil pH and only the highest ferrous sulfate further significantly reduced soil pH at a 2.5 cm depth. Ammonium sulfate and ferrous sulfate fertilizers' influence on soil pH was not a factor for annual bluegrass reduction in this trial. Repeated, high ferrous sulfate rates did not increase soil iron levels or result in a significant change in phosphorus or potassium soil levels after more than two years. However, the continuous application of SWE may retain soil phosphorus and potassium levels. Control of annual bluegrass in creeping bentgrass putting greens will continue to be a major challenge facing golf course superintendents. However, the seasonal biweekly foliar application of the medium rate of ferrous sulfate (24.4 kg $ha^{-1}$) safely and effectively reduced annual bluegrass infestation out of a creeping bentgrass putting green, while also effectively suppressing silvery thread moss incidence.

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## Appendix: Tables and Figures

**Table 1.** Analysis of variance for annual bluegrass infestation in a creeping bentgrass putting green as affected by ferrous sulfate rate, PGR (control, seaweed extract, paclobutrazol) or their combination over year.

| Treatment | Df | Annual Bluegrass |
|---|---|---|
| Year | 1 | NS |
| Ferrous Sulfate | 3 | * |
| Year x Ferrous Sulfate | 3 | NS |
| PGR | 2 | ** |
| Year x PGR | 2 | NS |
| Ferrous Sulfate x PGR | 6 | ** |
| Ferrous Sulfate x PGR x Year | 6 | NS |

**, and * = significant at $p < 0.01$ and 0.05, respectively. NS = Not Significant.
Plant Growth Regulator (PGR) = control, seaweed extract, paclobutrazol.

**Figure 1.** Interaction of ferrous sulfate rate and plant growth regulator on annual bluegrass infestation percentage on a creeping bentgrass putting green.

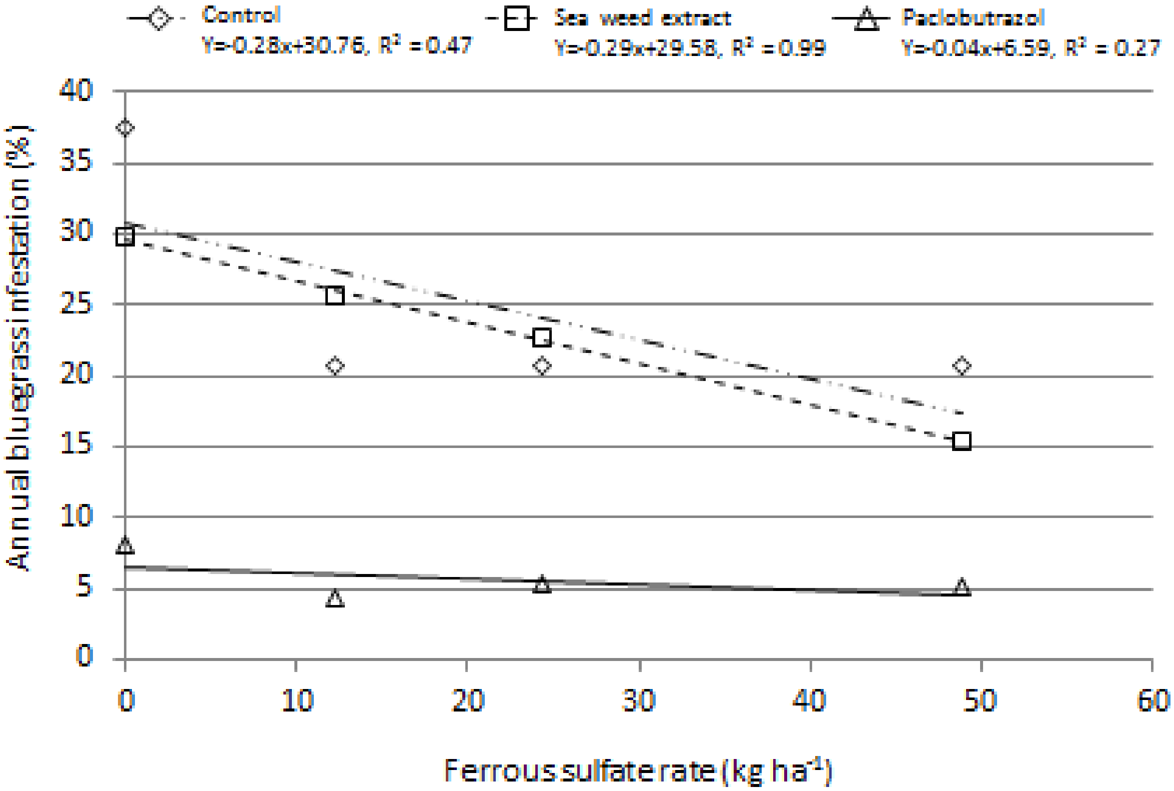

**Table 2.** Analysis of variance for turf color and quality as affected by ferrous sulfate rate, PGR treatment (control, seaweed extract, paclobutrazol), or their interaction on a creeping bentgrass putting green over year.

| | | Color | | | Quality | | |
|---|---|---|---|---|---|---|---|
| Source | Df | Spring | Summer | Fall | Spring | Summer | Fall |
| Year | 1 | NS | NS | NS | NS | NS | NS |
| Ferrous Sulfate | 3 | ** | ** | ** | ** | ** | ** |
| Year x Ferrous Sulfate | 3 | NS | * | ** | NS | NS | NS |
| PGR | 2 | NS | ** | * | NS | NS | NS |
| Year x PGR | 2 | ** | NS | NS | ** | * | NS |
| Ferrous Sulfate x PGR | 6 | NS | NS | NS | NS | NS | NS |
| Ferrous Sulfate x PGR x Year | 6 | ** | NS | NS | NS | NS | NS |

**, and * = significant at $p < 0.01$ and 0.05, respectively. NS = Not Significant.

**Figure 2.** Interaction of ferrous sulfate rate and plant growth regulator on turf color of an annual bluegrass infestation in a creeping bentgrass putting green.

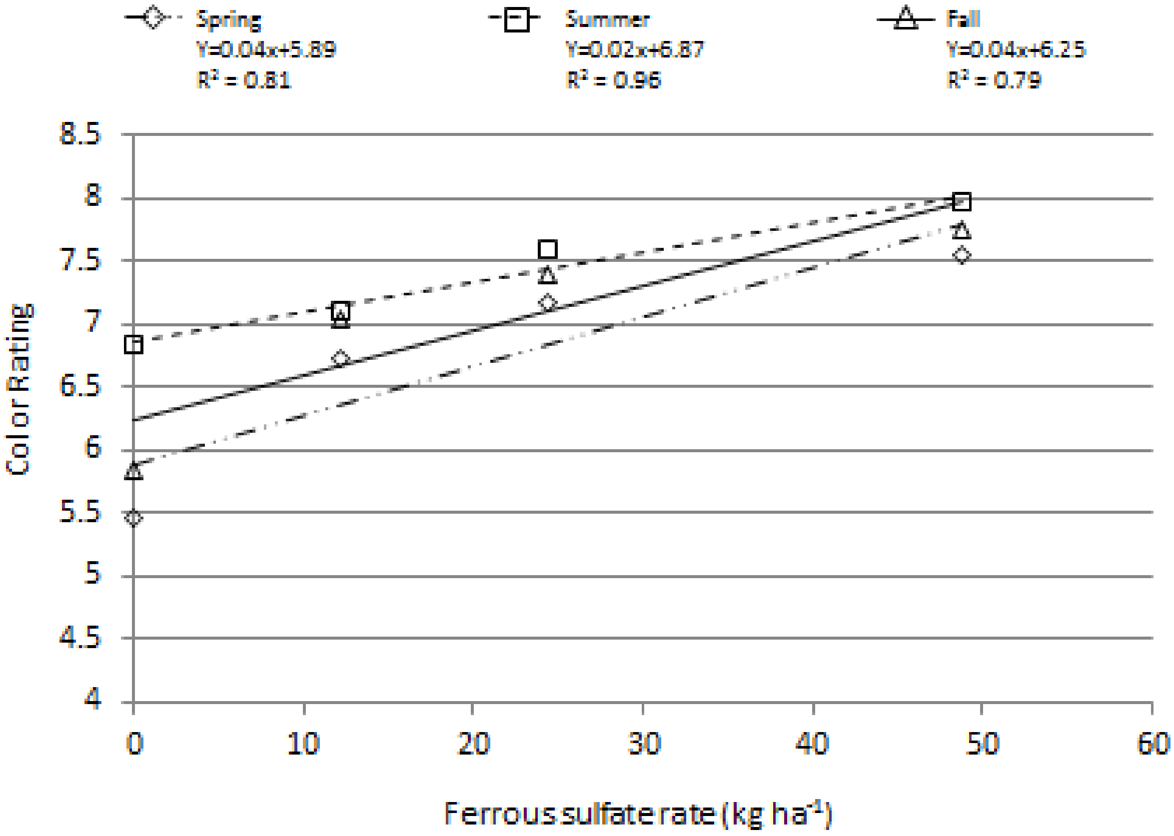

**Figure 3.** Interaction of ferrous sulfate rate and plant growth regulator on turf quality of an annual bluegrass infestation in a creeping bentgrass putting green.

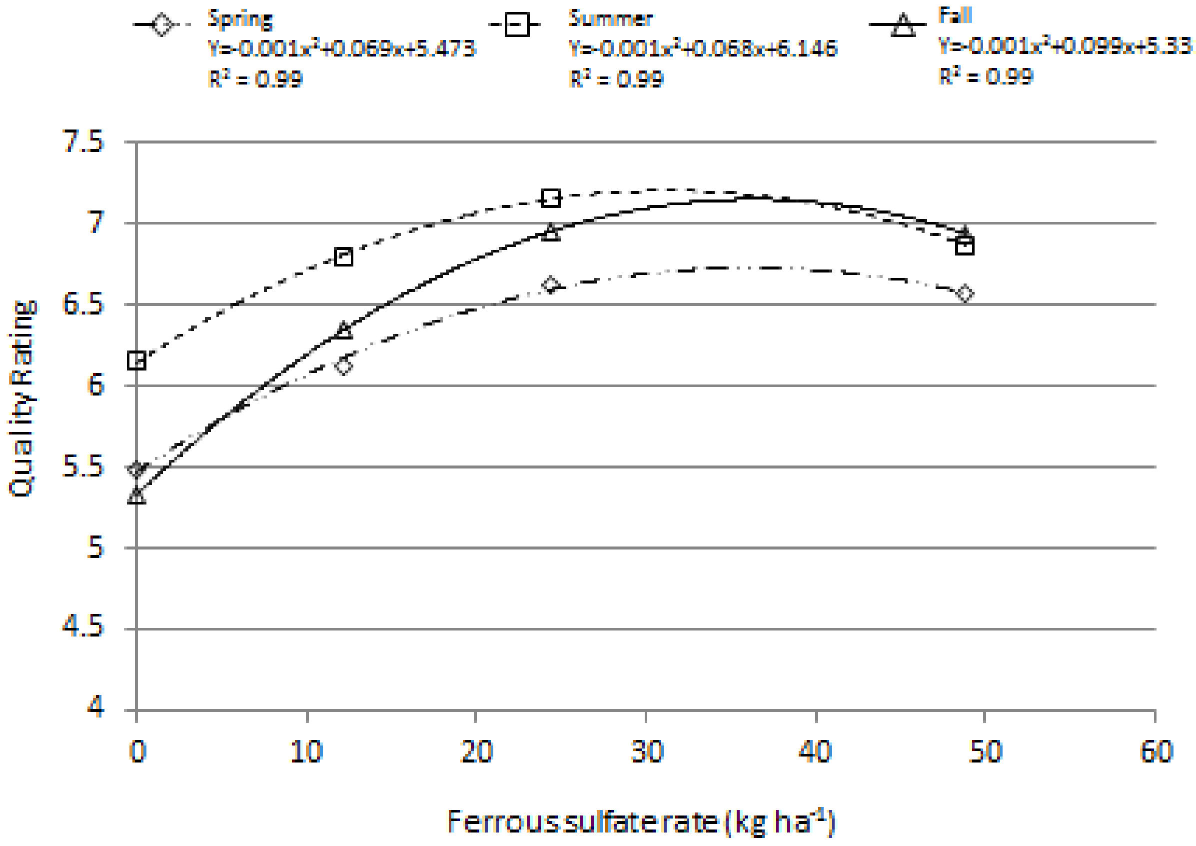

**Table 3.** Analysis of variance for soil pH, phosphorus, potassium, and iron at two depths as affected by ferrous sulfate rates, PGR treatments (control, seaweed extract or paclobutrazol) or their combination over year on a creeping bentgrass putting green.

| Source | Df | pH 2.5cm | pH 10.2cm | P 2.5cm | P 10.2cm | K 2.5cm | K 10.2cm | Fe 2.5cm | Fe 10.2cm |
|---|---|---|---|---|---|---|---|---|---|
| Year | 1 | ** | ** | NS | * | * | NS | ** | NS |
| Ferrous Sulfate | 3 | ** | NS | NS | NS | NS | NS | NS | ** |
| Year x Ferrous Sulfate | 3 | NS | NS | NS | NS | NS | NS | NS | NS |
| PGR | 2 | * | NS | * | NS | * | NS | ** | NS |
| Year x PGR | 2 | NS | NS | NS | NS | NS | NS | NS | NS |
| Ferrous Sulfate x PGR | 6 | NS | NS | NS | NS | NS | NS | NS | NS |
| Year x Ferrous Sulfate x PGR | 6 | NS | NS | NS | NS | NS | NS | NS | NS |

**, and * = significant at $p < 0.01$ and 0.05, respectively. NS = Not Significant.

**Figure 4.** Effect of ferrous sulfate rate on soil pH and iron levels averaged over PGR treatments and years as assessed at the end of each growing season.

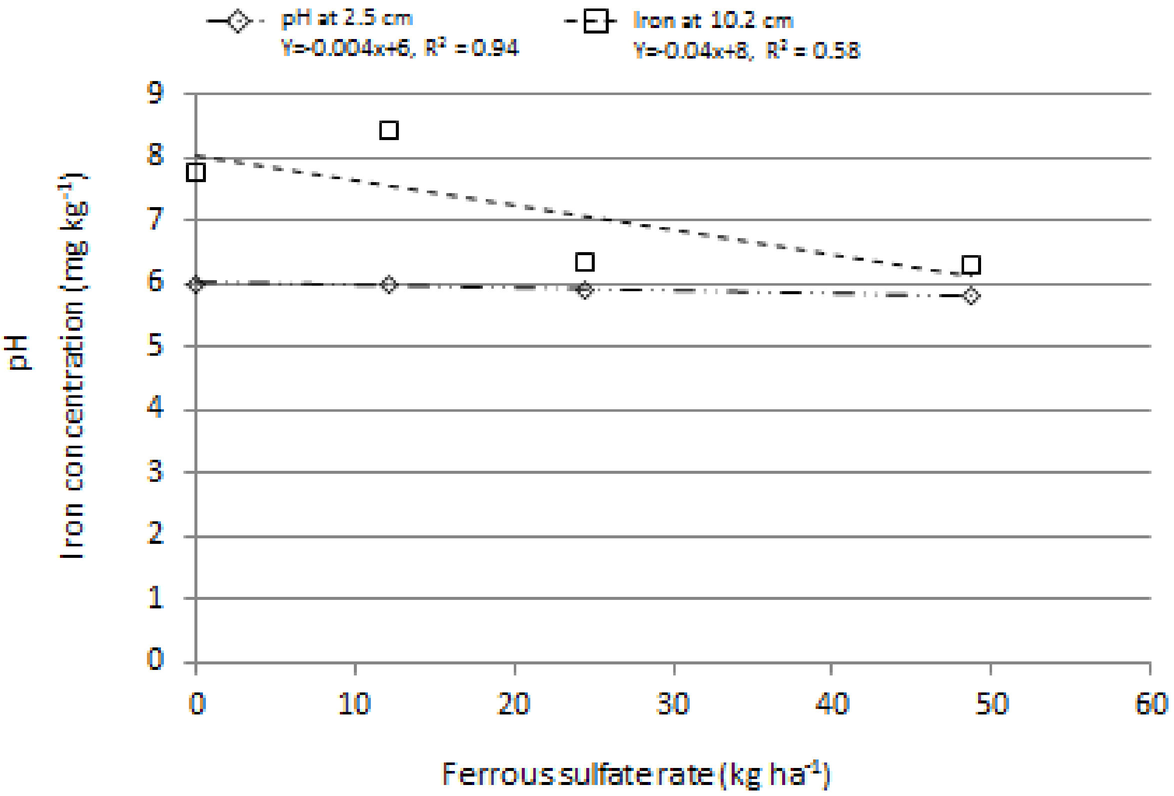

**Table 4.** Main effect of PGR treatments (control, seaweed extract, or paclobutrazol), averaged over year, on soil pH, phosphorus (P), potassium (K), and iron (Fe) levels at a soil depth of 2.5 cm on a creeping bentgrass putting green.

| PGR Treatments [y] | 2.5 cm Soil Depth | | | |
|---|---|---|---|---|
| | pH | P (mg kg$^{-1}$) | K (mg kg$^{-1}$) | Fe (mg kg$^{-1}$) |
| Control | 5.8b [z] | 4.3ab | 14.3b | 98.0a |
| Seaweed extract (12.8 L ha$^{-1}$) | 5.9a | 4.5a | 16.2a | 92.5ab |
| Paclobutrazol (0.37 L ai ha$^{-1}$ (Spring and Fall)) and 0.18 L ai ha$^{-1}$ (Summer)) | 6.0a | 3.9b | 14.9ab | 85.7b |

[y] Treatments were applied bi-weekly, March through October.

[z] Means in the same column followed by the same lower case letter are not significantly different according to Fisher's protected LSD test (P ≤ 0.05).

PGR = Plant Growth Regulator

**Table 5.** Analysis of variance for silvery thread moss in a creeping bentgrass putting green as affected by ferrous sulfate rate, PGR (control, seaweed extract, paclobutrazol) or their combination over year.

| Treatment | Df | Moss Count |
|---|---|---|
| Year | 1 | NS |
| Ferrous Sulfate | 3 | ** |
| Year x Ferrous Sulfate | 3 | * |
| PGR | 2 | ** |
| Year x PGR | 2 | NS |
| Ferrous Sulfate x PGR | 6 | ** |
| Ferrous Sulfate x PGR x Year | 6 | NS |

**, and * = significant at $p < 0.01$ and 0.05, respectively. NS = Not Significant.
Plant Growth Regulator (PGR) = control, seaweed extract, paclobutrazol.

**Figure 5.** Interaction of ferrous sulfate rate and plant growth regulator on moss colony counts on a creeping bentgrass putting green.

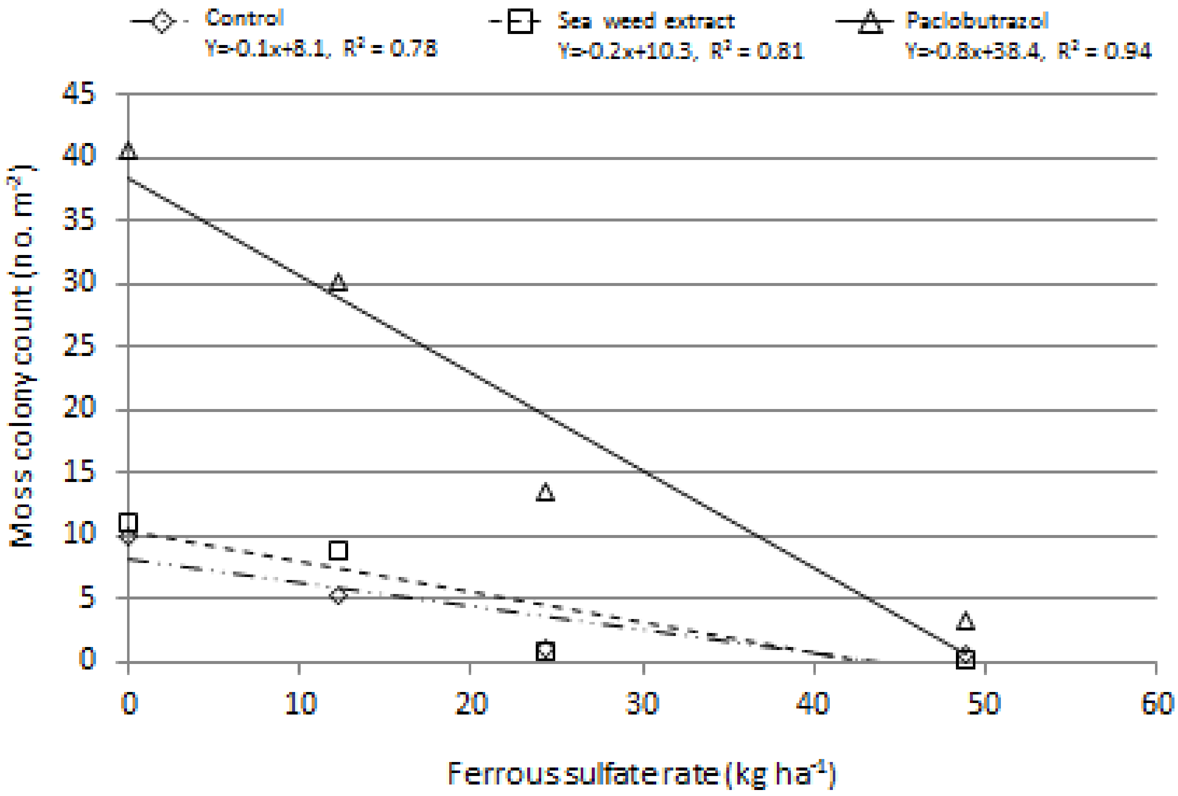

**Table 6.** Analysis of variance of dollar spot infection centers as affected by ferrous sulfate rate, PGR treatment (control, seaweed extract, paclobutrazol), or their interaction on a creeping bentgrass putting green in Fall 2011.

| Source | DF | Dollar Spot |
|---|---|---|
| Ferrous sulfate | 3 | **[y] |
| PGR[z] | 2 | * |
| Ferrous sulfate x PGR | 6 | NS |

[y] **, and * = significant at $p < 0.01$ and 0.05, respectively. NS = Not Significant

[z] PGR = control, seaweed extract, or paclobutrazol.

**Figure 6.** Dollar spot infection center count affected by the bi-weekly application of ferrous-sulfate on a creeping bentgrass putting green.

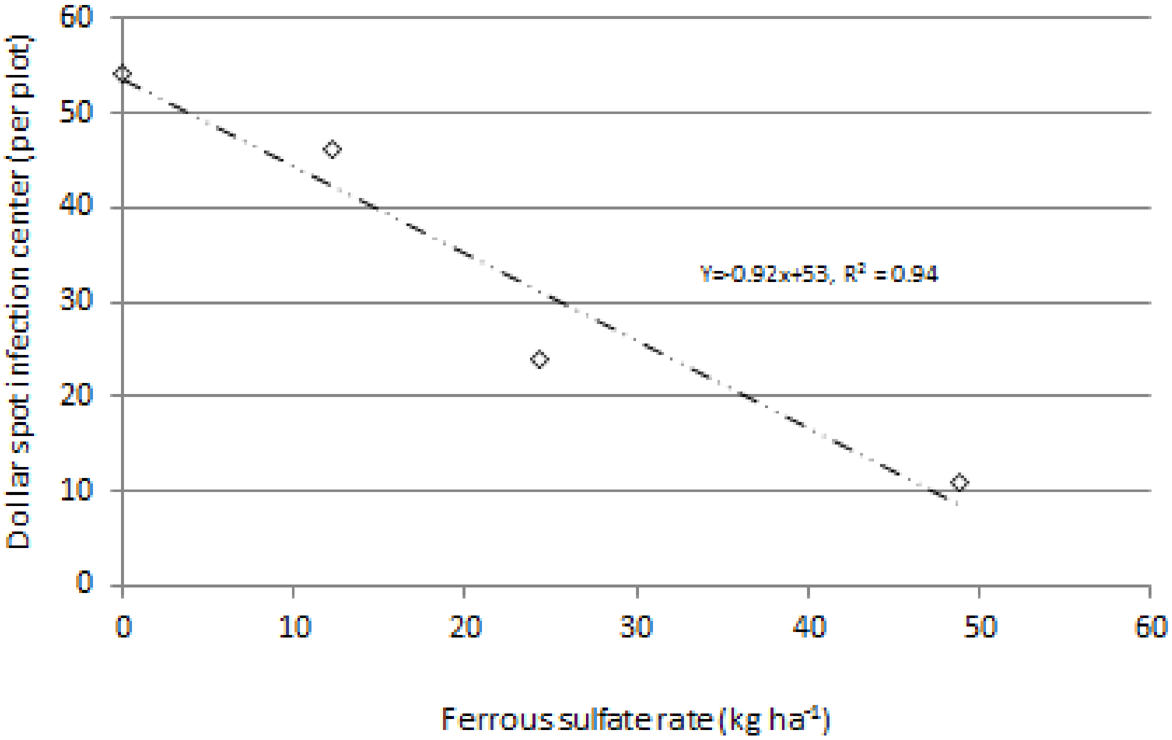

**Table 7.** Dollar spot infection center count affected by the bi-weekly application of seaweed extract and paclobutrazol on a creeping bentgrass putting green.

| PGR Treatments | Infection Centers |
|---|---|
| Control | 47.8a |
| Seaweed extract (12.8 L $ha^{-1}$) | 38.9a |
| Paclobutrazol (0.37 L ai $ha^{-1}$ (Spring and Fall)) and 0.18 L ai $ha^{-1}$ (Summer)) | 14.6b |

Means in the same column followed by the same lower case letter are not significantly different according to Fisher's protected LSD test ($P \leq 0.05$).

PGR = Plant Growth Regulator

**Figure 7.** Creeping bentgrass putting green turf quality as affect by paclobutrazol (0.37 kg ai ha$^{-1}$ (March 1 to June 1 and September 1 to October 31) and 0.18 kg ai ha$^{-1}$(June 1 to August 31)) and combination of ferrous sulfate rate (48.8 kg ha$^{-1}$) and paclobutrazol in July 2012.

**Figure 8.** Creeping bentgrass putting green turf color and quality as affect by the medium ferrous sulfate rate (24.4 kg ha$^{-1}$) in July 2012.

**Figure 9.** Voids in turf caused by annual bluegrass decline in a creeping bentgrass putting green affected by the highest ferrous sulfate rate (48.8 kg $ha^{-1}$) in August 2011.

**Figure 10.** A cohabited annual bluegrass and creeping bentgrass putting green black layer development as affected by the highest ferrous sulfate rate (48.8 kg ha$^{-1}$) in December 2012.

0.0 kg ha$^{-1}$ Ferrous sulfate

48.8 kg ha$^{-1}$ Ferrous sulfate

# Chapter 2

# The Influence of Iron, Sulfur, and pH on *Sclerotinia homoeocarpa* Infection of Creeping Bentgrass Putting Greens

## Introduction

Dollar spot (*Sclerotinia homoeocarpa* F. T. Bennett) is a destructive fungal pathogen of creeping bentgrass (*Agrostis stolonifera* L.) putting greens. *S. homoeocarpa* degrades bentgrass greens by creating silver dollar-sized (1-5 cm) depressions of dead turf and necrotic tissues that may persist throughout the winter and into the spring. To prevent disease symptoms, frequent fungicide applications are required throughout the growing season (Vargas, 2005). Even though many fungicides are labeled for dollar spot control, they have become limited due to government regulation, resistant fungal populations, or a failure to provide consistent control (Latin, 2012). Research into alternative methods for dollar spot control is required for improved integrated approaches to future disease control. Little published research has investigated claims that ferrous sulfate may act as a mild fungistat and may be an alternative control method for dollar spot (Arthur, 2003). For this reason we investigated the application of ferrous sulfate as a fungistat against *S. homoeocarpa*.

## Literature Review

Dollar spot was first described as “small brown patch” (Monteith and Dahl, 1932) and was described as small, sunken patches of blighted bentgrass turf as not larger than 5 cm in diameter or about the size of a silver dollar. As a result of this description the common name of dollar spot was given. In 1937, the dollar spot causal agent was identified as *Sclerotinia homoeocarpa* (Bennett, 1937). However, many mycologists now agree that the pathogen does not fit in the classification of *Sclerotinia* species, but further research will be need to taxonomically identify the appropriate species for the fungus (Vargas, 2005).

*S. homoeocarpa* mycelium is most often seen in the early morning when the dew is present. As the turf dries, the mycelium dries and disappears. Then infected blades of turf shrivel and turn to a bleached straw color. A closer look at the early stages of infection shows leaf tissue with tan to reddish brown lesions with margins having the shape of an hourglass. During mild epidemics, only the top section of the leaf blade may be affected, allowing the turf to recover from infection as the leaf tip is removed with daily mowing. If infection is allowed to continue, the infected grass will collapse and die, leaving a scar the size of a silver dollar in the turf. When this occurs, recovery of infected areas may take weeks (Monteith and Dahl, 1932; Smiley et al., 2005).

## Environmental Conditions

Environmental conditions are important factors for the pathogen to infect creeping bentgrass. Temperature, extended periods of leaf wetness, nitrogen availability, and soil moisture levels are factors that increase or decrease the potential for infection. Late-spring to early-summer weather conditions of high humidity, temperatures between 15-30°C, and turf canopy moisture for more than 10 hours favor pathogen mycelial growth (Smiley et al., 2005). In laboratory experiments active mycelial growth is seen between 5 and 10°C and begins to decline at temperatures higher than 30°C (Venu et al., 2009).

Warm, hot days followed by cool nights or improper irrigation timing in the mid- to late-morning or in the afternoon to early-evening extend periods of leaf wetness (Smiley et al., 2005). Cool nights during the summer produce morning dew and guttation water that contains carbohydrates and amino acids that are released through the hydathodes that increase the potential for infection (Vargas, 2005; Ellram et al., 2007). In the early-morning mycelium extend outward into the humid air and come into contact with a moist leaf surface. Then the aerial mycelium penetrates the leaf surface and infects the turf leaf blade (Smiley et al., 2005).

Water availability becomes an important issue during the summer months when creeping bentgrass needs for transpirational cooling increases. Irrigation practices should be utilized that reduce the period of leaf wetness and maintain adequate moisture for normal turf health. *S. homoeocarpa* infection has been shown to be more severe in late-summer on creeping bentgrass that received deep and infrequent versus light and frequent irrigation regimes (McDonald et al., 2006). When soil moisture fell near or below wilting point in late-summer, dollar spot became more severe under infrequent and deep irrigation. Low soil moisture levels occurring in late-spring and early-summer are not associated with increased *S. homoeocarpa* infection. Dollar spot is more active under conditions of low soil moisture and other summer stresses that weaken plant tolerance to the pathogen. Turf managed under low soil moisture grows more slowly and is less likely to recover from dollar spot damage. Higher soil moisture levels improved the dollar spot control efficacy of chlorothalonil and paclobutrazol (Pac) (McDonald et al., 2006).

Overall, plant health determines if turf is more or less likely to succumb to fungal infection. Nitrogen is an essential element for plant growth and development and sufficient levels of nitrogen increase overall plant vigor. As a result of sufficiently available nitrogen, the potential for dollar spot infection is reduced. Use of soluble nitrogen fertilizers have been observed to result in less dollar spot infection than turf not receiving any nitrogen (Markland et al., 1969). Turf receiving low levels of nitrogen fertility is more susceptible to *S. homoeocarpa* infection and requires more inputs of other control methods to maintain acceptable putting green playability (Smiley et al., 2005).

**Infection Process**

*S. homoeocarpa* infects turf by spreading and branching mycelium that penetrate turf leaves through the stomata or cuts in the blade from mowing (Smiley et al., 2005). Following cuticular penetration, the pathogen releases a toxin that has been associated with necrosis of leaf tissues and is able to infect the leaf blade. Venu et al. (2009) recorded that the mycelium associated with *S. homoeocarpa* produces oxalic acid as in accordance with other fungi belonging to the genus *Sclerotinia*. The acid produced by the pathogen is essential for pathogenicity. As a mycelium reaches a leaf blade, it produces oxalic acid to penetrate the leaf cuticle. Combining with calcium in the cell wall, oxalic acid leads to rapid degradation of pectic substances and weakens the cell wall, allowing the pathogen to enter the plant (Venu et al., 2010; Riou et al., 1991).

Oxalic acid is a simple organic acid that has a variety of toxic effects on plant cells and suppresses the oxidative burst associated with early host defenses (Walz et al., 2007; Hammerschmidt, 2007). In some host-pathogen interactions, oxalic acid suppresses plant defense mechanisms, whereas in others, oxalic acid produced by the pathogen functions to exploit plant defenses for its own growth and ability to spread in the host tissue (Walz et al., 2007). *S. homoeocarpa* infection of creeping bentgrass was observed to increase oxalic acid at the infection site, leading to degradation of turf health (Hammerschmidt, 2007).

Oxalic acid production has been correlated with abundant mycelial growth which peaked at temperatures between 20 and 30$^{o}$C, with declines in growth above and below these temperatures (Venu et al., 2009). As the acid production increases, it induces foliar wilting by causing a water potential change in the guard cells. Stomatal pores are partially closed when leaves are infected and as the acid spreads, it causes an increase in stomatal conductance and transpiration. Increased oxalic acid interferes and inhibits abscisic acid for inducing stomatal closure and even prevents stomatal closure in the dark. The fungus exploits the open stomatal pores for hyphal emergence and secondary colonization. All stomatal pores in a 5 mm diameter around necrotic lesions have been reported to be open as a result of pathogen infection (Guimaraes and Stotz, 2004).

### Dispersion

The *S. homoeocarpa* colonizes the root crown region and turfgrass canopy where it produces mycelia that is dispersed by equipment, people, animals, water, or wind. Although the pathogen does not disseminate via sexual or asexual spores, dispersion is most likely attributed to frequent mowing by the same mower (Jo et al., 2008; Horvath et al., 2007). Infection may first occur on the side of the green where golf and maintenance traffic enters, possibly from infected approaches and fairways (Vargas, 2005).

The *S. homoeocarpa* population structure was different on turfgrass sites managed differently, suggesting that unique management practices employed on various turfgrass sites affect fungal populations. Mowing frequency and height, and chemical inputs, are more intense on putting greens than fairways and roughs, creating diversity among the *S. homoeocarpa* isolates found at both locations. Two *S. homoeocarpa* subgroups are found between greens and fairways that are genetically different, vegetatively incompatible, and differing in fungicide sensitivity. Within each area of the golf course the two different subgroups are spread uniformly across each area. Isolates within the same subgroup produced stable hyphal anastomoses, functioning to ensure potential genetic exchange and diversity build up (Jo et al., 2008).

The *S. homoeocarpa* is often introduced at turf establishment, during which time populations may arise from small populations or a single strain (Beard and Beard, 2005). As time goes on the population becomes more persistent as it becomes established. After establishment, seasonal weather conditions influence the pathogen population size and ability to disperse and increase in size. During cold winter months the silver dollar size spots fade out, but during the spring and dry summer months the spots redevelop as new infection, with dispersion increasing into the fall (Smith, 1959). The fungus survives in infected plants and plant debris as mycelium and on the leaf surface as stromata during unfavorable environmental conditions for development. When environmental conditions favor fungal activity, mycelia within previously infected tissue or from stromata colonizes the foliage (Smiley et al., 2005).

### Control Methods

To meet the high expectations for putting green aesthetics and playability, fungicide inputs are required for managing dollar spot on creeping bentgrass and annual bluegrass greens. Repeated fungicide applications are required throughout the growing season to suppress *S. homoeocarpa* infection. With increasing levels of fungicide resistance and tighter regulations for existing fungicides, fewer chemical options are available for controlling dollar spot. The current list of registered fungicide classes that are most effective at controlling dollar spot are as follows: aromatic hydrocarbons, benzimidazoles, carboxamides, nitriles, QoIs, dicarboximides, DMIs, and phenylpyrolles (Latin, 2012).

The FQPA (Food Quality Protection Act) of 1996 reduces the total amount of allowable risk for pesticides, providing safer standards for pesticide use. Even though turfgrasses are not grown for human consumption, FQPA has placed restrictions on pesticide use, reducing the number of available pesticides for controlling turf pests. For example, chlorothalonil and thiophanate-methyl fungicides were re-registered in 1996, placing restrictions on the amount of active ingredient that is allowed to be applied each year. The FQPA limited the use of multi-site contact fungicides, such as chlorothalonil, forcing turf managers to rely on site-specific inhibitors that are more prone to resistant pathogenic populations (Latin, 2012).

Because *S. homoeocarpa* is a prolific mycelial producer, consistent fungicide applications are required to keep populations low, increasing the potential for resistant populations (Latin, 2012). Demethylation inhibitors (DMI) fungicides provide control for more extended periods of time and are effective at post infection control. Dollar spot resistance to DMI fungicides has developed in recent years and is attributed to their repeated application (Jo et al., 2008). The DMI fungicide class and class B plant growth regulators (PGR), Pac and flurprimidol, share triazole and pyrimidine chemical structures that target sterol biosynthesis. Flurprimidol and Pac applied consecutively or tank-mixed with a DMI increases dollar spot control, turf quality, and the selection pressure leading to a field resistant population (Bishop et al., 2008; Burpee, 2001; Fidanza et al., 2006; Golembiewski et al., 1995; Miller et al., 2002; Ok et al., 2011).

As dollar spot resistance to DMI fungicides occurs, a reliance on single-site mode-of-action fungicides such as the benzimidazole and carboxamide classes is likely to occur. Single-site fungicides require only a single mutation for that mode-of-action to become ineffective for dollar spot control. Multiple resistant populations to benzimidazoles, dicarboximides, and carboxamides have been observed and will likely increase in the future as reliance increases (Ok et al., 2011; Golembiewski et al., 1995; Burpee, 1997). Thiophanate-methyl, a benzimidazole class fungicide, has also been reported, with repeated use, to rapidly result in the development of highly resistant populations that become dominant in the dollar spot population (Burpee, 2001; Jo et al., 2008; Bishop et al., 2008).

Chlorothalonil, a nitriles fungicide class, can provide acceptable levels of dollar spot control in creeping bentgrass turf, but requires frequent and high application rates for consistent control. The rate and application intervals for this fungicide are restricted, limiting its use as a main fungicide for dollar spot control. Because chlorothalonil is a multi-site contact fungicide, the development of resistance requires more than one mutation in the population to occur (McDonald et al., 2006).

**Cultivar**

As an alternative method for dollar spot management, creeping bentgrass has been bred for reduced sensitivity. Among the many creeping bentgrass cultivars used for turf, susceptibility to dollar spot varies among the cultivars (Lee et al., 2003). Creeping bentgrass resistance to *S. homoeocarpa* infection can be attributed to two possible causes: the size of the trichome (Bonos et al., 2004) and the suppression by the cultivar of oxalic acid activity produced by the *S. homoeocarpa* (Hammerschmidt, 2007).

A possible reason for varying susceptibility among creeping bentgrass cultivars is related to the size of the trichome found on the leaf surface. Cultivars with larger trichomes are more resistant to pathogen infection than those with smaller trichomes. The trichome acts as a physical hindrance preventing the mycelium from reaching the host (Bonos et al., 2004).

Recently developed cultivars of creeping bentgrass that have been shown to be relatively dollar spot resistant because of increased oxalic acid oxidase activity during pathogen infection (Hammerschmidt, 2007). The oxalate oxidase or oxalate decarboxylase converts oxalic acid into carbon dioxide and formate leading to delayed pathogen growth in the host tissue. Under normal growth conditions the production of reactive oxygen intermediates is low, but under attack by oxalic acid producing pathogens reactive oxygen species may remain low. Plants that increase levels of reactive oxygen intermediates activate several plant defense mechanisms, thus delaying the fungal growth. An additional secretion of hydrogen peroxide reduces pathogen growth (Walz et al., 2007).

Even though some creeping bentgrass cultivars are partially or highly resistant to *S. homoeocarpa* infection, other control methods are needed to provide acceptable control during periods of high disease pressure. Bentgrass cultivars with low resistance to dollar spot infection require increased fungicide applications, even during periods of low disease pressure. Highly resistant cultivars require fewer fungicide inputs, even during periods of high disease pressure (Settle et al., 2001).

### Cultural Control Methods

Cultural methods are part of an integrated approach for controlling dollar spot. However, cultural practices alone are not effective at controlling dollar spot, but can increase fungicide application longevity and control. Previous research has investigated nitrogen source for dollar spot control (Markland et al., 1969), but little research has investigated the effects of sulfur, iron, or ferrous sulfate on control of dollar spot or other turf pathogens.

As early as 1824, elemental sulfur was the first fungicide applied to plant foliage to control powdery mildew (*Blumeria graminis* (DC.) E. O. Speer) (Maloy, 1993). Frequent sulfur applications to control dollar spot have been reported to be ineffective and may even encourage mycelial growth (Monteith and Dahl, 1932). In contrast, when sulfur was used to counteract the effects of alkaline irrigation water, dollar spot infection centers were reduced in mid-spring. Sequential applications of sulfur did not have an effect on dollar spot infection compared to no sulfur applications the following months of the year. The authors believed that reduced *S. homoeocarpa* infection can be attributed to insufficient sulfur levels in the soil before the trial was initiated and the sulfur application increased soil sulfur levels and turf health (Bell et al., 2001).

Elemental sulfur fertilizer applications are oxidized in the soil by *Thiobacillus* species into sulfuric acid. Once the sulfuric acid is formed it can easily disassociate, releasing hydrogen ions that lower soil pH (McCarty et al., 2003). The sulfur application effect of lowering soil pH has been observed not to have an effect on *S. homoeocarpa* infection (Smiley et al., 2005). This result was also observed in laboratory experiments when no significant differences were found in *S. homoeocarpa* mycelial growth on a media with pH ranging from 4 to 7 (Smith, 1959; Bennett, 1937; Venu et al., 2009).

Even though pH was not a factor for mycelial growth in previous studies, a more recent study reported that increased oxalic acid and mycelial production in acidic media occurred when compared to growth in an alkaline media (Venu et al., 2009). They further reported that oxalic acid production by the pathogen is pH-dependent occurring 24-48 hours earlier at pH 6 compared to pH 4. At 13°C, the amount of aerial mycelia was greater on the acid side and less on the alkaline side. Acid conditions are more favorable to the rate and vigor of mycelial growth than alkaline conditions (Bennett, 1937). Soil acidification may be a factor in dollar spot suppression on isolates located in Maryland. It was hypothesized that soil acidification affects the microbial populations found in the soil resulting in dollar spot suppression (Ryan, 2011).

When activated sewage sludge was used on creeping bentgrass it significantly increased iron, copper, and zinc leaf tissue content. As a result, the incidence of dollar spot was reduced. The authors of the experiment hypothesized that uptake of mineral elements such as iron, copper, and zinc from activated sewage sludge may result in fungitoxic accumulations and prevent dollar spot infection (Markland et al., 1969).

Iron can assist in the suppression of pathogens that infect plants or it can aid in the infection process of certain pathogens (Forsyth, 1957; Graham, 1983). Iron deficiency in wheat (*Triticum aestivum* L.) has been shown to cause a breakdown in pathogen resistance, while iron application was shown to cause pathogen resistance in normally susceptible plants. The combination of iron and sulfur as ferrous sulfate has been shown to decrease or suppress the infection of rust (*Puccinia graminis* Pers.) in wheat at concentrations of 40 to 200 mg iron $kg^{-1}$. The rust hyphae are killed and the infection areas become dark brown to black. At the site of infection, iron ions were found to be concentrated leading the author to hypothesize that the metabolism of iron may play a role in defense against potential pathogens (Forsyth, 1957). Iron can have the reverse effect on certain pathogens. The *Fusarium* (*Fusarium acuminatum* Ellis & Everh.) pathogen requires iron for the production of pectin methylesterase, which is used by the pathogen to attack the middle lamella pectin enabling the pathogen to invade the plant (Graham, 1983).

Repeated, high application rates of ferrous sulfate (48.8 kg $ha^{-1}$) have been observed to control or reduce dollar spot infection on creeping bentgrass putting greens (Reams, personal observations). Very little literature is available on the effects of iron or sulfur on turf fungal pathogens other than that sufficient levels in turfgrass allow for normal health and growth, reducing possible infection. It is unknown if higher rates of iron, sulfur, or ferrous sulfate are toxic to the *S. homoeocarpa* or hinder the *S. homoeocarpa* infection process. For these reasons, this study investigated which element or elements of ferrous sulfate are responsible for dollar spot control in creeping bentgrass putting greens.

## Materials and Methods: Field Study

A field trial was conducted at the Virginia Tech Turfgrass Research Center in Blacksburg, VA, between March and September 2012 and was arranged in a randomized complete block design. Individual plots measured 1.8 m x 1.8 m with five replications of each treatment. The trial was conducted on a mature 'Penn A-4' creeping bentgrass putting green with a history of heavy dollar spot infestation. The green was built to USGA specifications (90% sand, 10% peat moss) and had an initial soil pH of 6.3 at 2.5 cm and 10.2 cm depths. Initial iron concentrations were 102.7 mg kg$^{-1}$ at 2.5 cm and 120.5 mg kg$^{-1}$ at 10.2 cm depth, which are considered high for management of creeping bentgrass (McCarty et al., 2003). The green was mowed five times a week at 3.2 mm and clippings were removed. The plot area was core aerified and sand top dressed in the spring and fall, removing 15% surface area for the year. The plots were also solid tined every three to four weeks during the summer months. Irrigation water (75 mL) was collected and a pH meter (VWR Scientific Products SR 601C, Radnor, PA) was used to determine the pH to be 7.6; the green was irrigated as needed to prevent visual signs of wilt. Nitrogen was applied weekly or biweekly in the form of urea at a rate of 7.3 kg N ha$^{-1}$ totaling 146.0 kg N ha$^{-1}$ yr$^{-1}$ (see Table 1 for details).

Ferrous sulfate (19% iron and 21% sulfur, Hi-Yield®, Bonham, Texas) and its elemental components were investigated for their effects on dollar spot epidemics. Sulfur and iron rate were determined by the percentage of each element in 48.8 kg ha$^{-1}$ of ferrous sulfate, using 90% elemental sulfur (Hi-Yield®, Bonham, Texas) and 10% chelated iron (iron ethylenediaminetetraacetic acid (EDTA), Sprint®, Ames, IA). All treatments were applied bi-weekly as liquids with a $CO_2$-pressurized (275.6 kPa at 374 L ha$^{-1}$) walk behind sprayer with XR Tee Jet 8003VS nozzles. Treatments were applied in a randomized complete block design as follows: control, sulfur at 10.3 kg $ha^{-1}$, ferrous sulfate at 48.8 kg $ha^{-1}$, and Fe-EDTA at 11.2 kg iron $ha^{-1}$.

Dollar spot counts were conducted on four occasions when control plots were infested with approximately 25 to 30 infection centers at the end of each cycle. A cycle includes a recovery period, dollar spot infection period and day of counting. Counts were done using golf tees to mark the location of each individual infection center in the early morning when dollar spot mycelia were observed. Following data collection, plots were treated with fungicides and weekly nitrogen applications (Table 1) and allowed to fully recover (no symptom expression). Following recovery, fungicides were discontinued and nitrogen frequency was reduced to bi-weekly to encourage the development of a new dollar spot epidemic. Cycles typically lasted six to seven weeks, depending on weather conditions.

Quality was rated one week after each of the bi-weekly treatments from March through September. Quality ratings were based on a 1-9 scale with 1 = dead, poor quality turf, 6 = minimally-acceptable turf, and 9 = healthy, high quality turf. The bi-weekly quality ratings that occurred between the beginning and end of each disease cycle were averaged to control for variance structure in the repeated measures. The disease cycle periods were chosen because quality seemed to be seasonal in response and could be better compared with disease data.

Soil samples were collected at 2.5 cm and 10.2 cm depths to determine pH and iron levels at trial initiation and conclusion. Soil analysis was performed by the Virginia Tech Soil Testing Lab located in Blacksburg, VA using Mehlich buffer solution and following Mehlich I extraction procedures (Maguire and Heckendorn, 2011).

Data were subjected to analysis of variance (ANOVA) using the general linear models procedure in SAS, version 9.1 (SAS Institute, Cary, NC). If treatment effects were significant, means were separated with Fisher's protected LSD test ($\alpha = 0.05$).

## Results: Field Study

### Dollar Spot Infection Center Counts

The ANOVA test for dollar spot infection centers indicated a significant effect of fertilizer treatment during three of four cycles (Table 2). Dollar spot infection centers did not differ between treatments during the first disease cycle, which was characterized as having moderately-low dollar spot pressure (Table 3). By the end of the second cycle, plots treated with ferrous sulfate and Fe-EDTA had less dollar spot infection centers compared to the control and sulfur treatments (Table 3). This trend of reduced dollar spot due to the iron treatments was also observed during the remaining two disease cycles. Ferrous sulfate reduced dollar spot to less than one infection center per plot by the third cycle count, but was not significantly different from Fe-EDTA. Elemental sulfur had no effect or increased dollar spot infection centers relative to the control during the four cycles.

### Quality

Creeping bentgrass turf quality was significantly affected by fertilizer during all but the first disease cycle (Tables 2 and 3). By the end of cycle two, ferrous sulfate and Fe-EDTA improved turf quality compared to the control and elemental sulfur. As the trial continued, ferrous sulfate continued to improve turf quality while Fe-EDTA decreased quality. By the end of the trial, Fe-EDTA negatively affected turf quality, reducing it to below acceptable levels.

### Soil pH and Iron

Soil pH and iron levels at 2.5 and 10.2 cm depths were not influenced by fertilizer treatments (data not shown). At study completion, pH was 6.0 at both soil depths while iron was 124 and 162 mg $kg^{-1}$ at the 2.5 and 10.2 cm depths, respectively.

## Discussion: Field Study

Throughout the trial sulfur had no effect on turfgrass quality, relative to the control. Ferrous sulfate, however, improved quality starting at the second disease cycle and continuing through the remainder of the trial. Through cycle two, Fe-EDTA also provided the best quality, and then declined to an unacceptable level by cycle four. Turf quality decline in the Fe-EDTA plots occurred due to what appeared to be an accumulation of iron at the soil surface (a black layer) that caused a 20 to 30% loss of turf cover by the end of cycle four (see Figure 5). Fe-EDTA applications are recommended every three to four weeks at 3 to 6 kg $ha^{-1}$, depending on desired color. If application rates are higher and more frequent than these recommendations, Fe-EDTA has the potential to become toxic to creeping bentgrass (McCarty, 2005).

Xu and Mancino (2001) reported that iron citrate applied at 10.1 kg iron $ha^{-1}$ every other day for three weeks reduced creeping bentgrass root and shoot growth. The authors surmised that creeping bentgrass decline following this rate and frequency of iron citrate was due to iron toxicity. In the present trial, Fe-EDTA was applied at two to four times the recommended safe rate (11. 2 kg iron $ha^{-1}$ every two weeks) (McCarty, 2005) and most likely delivered iron concentrations that were also toxic to creeping bentgrass. The decline in turf coverage became evident after daily high temperatures averaged 35$^{o}$C. Ferrous sulfate and sulfur did not decrease turf coverage during this period.

Fe-EDTA is recommended for acid soils because the iron chelate is stable under acidic conditions (Havlin et al., 2005). Putting green soil in this study was pH 6.0, but irrigation water was pH 7.6

and may have caused the chelate to become unstable as the irrigation water infiltrated the soil. This sudden release of iron may have led to creeping bentgrass collapse during the summer as the plots were irrigated more frequently. Fe-EDTA slowly releases ferric iron while ferrous sulfate quickly releases ferrous iron (Havlin et al., 2005). Iron may be accumulating at the soil surface as Fe-EDTA allowing for toxic levels of ferric iron release with each irrigation event. Since ferrous iron is more soluble than ferric iron (Havlin et al., 2005), ferrous sulfate does not likely accumulate on the soil surface but rather leaches into the soil column and is diluted or carried away in drainage water. Superintendents have reported compromised drainage systems due to iron accumulation in pipes following continuous use of ferrous sulfate (Obear, 2013).

At the end of the first cycle, fertilizer treatments did not significantly affect dollar spot but differences were noted later in the season (Table 3). After the first cycle, ferrous sulfate and Fe-EDTA reduced dollar spot while sulfur did not. Since dollar spot was only reduced by the two iron-containing treatments, iron is most likely the cause for reduced dollar spot infection centers (see Figure 6). However, iron applied as a chelate at high rates reduced turf quality over time, while the ferrous sulfate increased quality over time.

Forsyth (1959) observed the suppression of rust in wheat with the application of ferrous sulfate. The application of ferrous sulfate increased the iron concentration in wheat tissue and suppressed the rust pathogen infection. The author concluded that the iron ions killed the rust hyphae and prevented infection. It is hypothesized that the ferrous sulfate and Fe-EDTA had to be applied long enough to build up sufficient iron levels in the thatch and turf tissue for these fertilizers to become effective at suppressing dollar spot infection. Further experimentation is needed to test this supposition.

Monteith and Dahl (1932) observed an increase in dollar spot activity as a result of elemental sulfur fertilizer applications. For the third cycle's dollar spot infection center count, elemental sulfur was observed to significantly increase dollar spot infection centers compared to untreated check and other fertilizers. Little information is available as to why sulfur may increase dollar spot activity.

Lack of soil pH variation among fertilizer treatments can probably be attributed to two factors. First, the slightly alkaline irrigation water contained 12 mg $kg^{-1}$ calcium and may have counteracted the potential acidifying effects of sulfur and ferrous sulfate. The bicarbonate and carbonate react readily with calcium to form calcium carbonate, which has a low solubility and can decrease the effectiveness of sulfur to reduce soil pH (Carrow and Duncan, 1998). The second factor that may have reduced soil pH variability is that downward movement of oxidized sulfur into the root zone is slow and may lead to a dramatic pH decrease in the thatch layer with minimal effect on the root zone (McCarty et al., 2003).

A possible explanation for iron levels having no significant variation among the fertilizer treatments is that the thatch layer was removed prior to soil analysis. Visual examination of a soil core sample taken from the ferrous sulfate and Fe-EDTA treatment plots showed a black layer that had developed in the thatch layer (Reams, personal observations). This black layer may have been caused by the iron from ferrous sulfate and Fe-EDTA fertilizers. Elemental sulfur and untreated soil core samples did not have a visible black layer in the thatch. The accumulation of iron in the thatch layer can be minimized by frequent core aerification and sand topdressing practices as recommended for thatch control (McCarty, 2005).

## Materials and Methods: *In-vitro* Study

Isolated cultures of *Sclerotinia homoeocarpa* F.T. Bennett were collected from a creeping bentgrass (‘Penn A4’ *Agrostis stolonifera* L.) putting green at the Virginia Tech Turfgrass Research Center in Blacksburg, VA. Infected leaf samples were surface sterilized with 10% bleach (6% sodium hypochlorite) for 10 seconds and rinsed. Leaves were placed on ¼ strength potato dextrose agar (PDA, Bacto, Difco Laboratories, Detroit, MI) where mycelia were observed for growth and transferred from contamination free areas of the petri dish to a separate petri dish. Morphological characteristics of pure colonies consistent with *S. homoeocarpa* (Smiley et al., 2005) were selected. Sample isolate ‘TRC 2C-2’ was chosen for the experiment.

Ferrous sulfate (19% iron and 11% sulfur, Hi-Yield®, Bonham, Texas) was used as the iron source and was put into solution using sterile deionized water. This solution was then added to ¼ strength PDA after being autoclaved. After the fertilizer addition, 0.10 mL of lactic acid was added to each PDA solution to ensure the fertilizer did not cause any contamination of the PDA. Once the various concentrations of iron were added to the PDA, pH was adjusted to each treatment pH using diluted ammonia hydroxide or lactic acid at 10:1 and 100:1 ratios, respectively. The pH was tracked using pH meters (VWR Scientific Products SR 601C, Radnor, PA) in the PDA. Levels of pH in the PDA that were tested were as follows: 4.5, 5.0, 5.5, and 6.5; all of these pH levels were tested in combination with the following iron concentrations: 0, 10, 100, and 1000 mg $kg^{-1}$.

A 2 mm disk of mycelia was placed upside down on the solidified PDA. Samples were maintained in the dark at ambient air temperature (20-22°C) and randomly stacked. Blank PDA plates were used as checks for each treatment to track any bacterial or other microbial contamination. No contamination was observed during the trial periods. After three days, mycelial growth diameters were measured in mm and recorded. First the mycelial diameter was measured in one direction and then the petri dish was rotated 90 degrees and a second measurement was taken. An average of the two measurements was recorded. The project was repeated twice to verify that results were consistent.

Two trials, each with five replications, were conducted as a completely randomized design with pH (4.5, 5.0, 5.5, 6.5) and iron concentration (0, 10, 100, 1000) in a factorial arrangement. Data were subjected to a combined analysis of variance (ANOVA) using the general linear models procedure in SAS, version 9.1 (SAS Institute, Cary, NC) with sums of squares partitioned to reflect the factorial treatment design and trial effects, which were considered random. Mean squares were tested as appropriate for the factorial design and random trial (McIntosh, 1983). Interactions and main effects were separated with Fisher's protected LSD test ($\alpha = 0.05$) or described with polynomial regression where appropriate.

## Results: *In-vitro* Study

The ANOVA test for *S. homoeocarpa* radial mycelial growth as affected by agar pH and iron concentration indicated a significant interaction between agar pH, iron concentration, and trial. The ANOVA table is shown in Table 4. Due to the trial interaction, data for each trial will be presented separately.

### Effects of iron concentration at varying pH

The interaction of agar pH and iron concentration is shown as regressions of iron concentration at each level of pH for trial 1 and 2 in Figures 1 and 2, respectively. In trial 1 at pH 4.5 and 6.5, iron concentrations between 0 and 100 mg kg$^{-1}$ increased radial mycelial growth but 1000 mg kg$^{-1}$ iron suppressed mycelial growth (Figure 1). At pH 5.0 and 5.5, iron concentrations of 100 mg kg$^{-1}$ or less did not affect mycelial growth but 1000 mg kg$^{-1}$ iron decreased mycelial growth

(Figure 1). In trial 2 regardless of pH, the curvilinear response of iron concentrations on mycelial growth was more evident, showing a slight increase between 0 and 10 mg $kg^{-1}$ and a decrease as iron concentration increased to 100 mg $kg^{-1}$ or more (Figure 2).

### Effects of pH at varying iron concentrations

The interaction of agar pH and iron concentration is shown as regressions of pH level at each iron concentration for trial 1 and 2 in Figures 3 and 4, respectively. In trial 1, radial mycelial growth exhibited a curvilinear response across pH, with optimal growth at 5.4 pH (Figure 3). At 10 mg $kg^{-1}$ iron, mycelial radial growth decreased with increasing pH (Figure 3). In contrast, increasing pH positively affected mycelial growth at 100 mg $kg^{-1}$ iron (Figure 3). Regardless of pH level, 1000 mg $kg^{-1}$ iron suppressed mycelial radial growth (Figure 3). In trial 2, regardless of iron concentration, increasing pH either decreased or had a minimal impact on mycelial radial growth (Figure 4).

## Discussion: *In-vitro* Study

Previous *in vitro* studies have indicated that agar pH does not have an effect on dollar spot mycelial growth. Differences in mycelial growth have been observed during these studies among the acidic pH ranges, but were not statistically different (Smith, 1959; Bennett, 1937; Venu et al., 2009). This experiment indicated that agar pH had a significant influence on dollar spot mycelial radial growth on isolates collected in Blacksburg, VA. The dollar spot isolates used for this study were collected from a research putting green that has been subjected to varying research treatments for the past few years. These varying treatments at the site of collection may have produced an adapted dollar spot species that may react differently than isolates collected from a golf course or other locations (Jo et al., 2008).

A general trend observed for both trials indicates an increase in growth of *S. homoeocarpa* at 10 mg iron kg$^{-1}$ concentration, regardless of agar pH. The exogenous application of iron at 55 mg kg$^{-1}$ has been observed to increase fungal pathogenicity into a plant host. The pretreated application of iron at the same concentration increased the potential for fungal pathogenicity, suggesting that readily available iron can potentially increase fungal growth (Oide et al., 2006). However, high concentrations of iron may be toxic or prevent a fungal infection as observed in this *in vitro* study. The 1000 mg iron kg$^{-1}$ concentration was observed to have the smallest mycelial diameters for both trials indicating that a significant concentration of iron ions reduces or prevents growth.

In addition to a direct fungitoxic effect on *S. homoeocarpa*, iron may also chelate with oxalic acid to disrupt pathogenicity. Venu et al. (2009) observed oxalic acid production by the dollar spot pathogen as the main compound for pathogenicity. Oxalic acid is a known chelating agent for iron ions (Havlin et al., 2005). The prevention of the oxalic acid activity by *S. homoeocarpa* may prevent pathogenicity and infection into leaf tissue (Hammerschmidt, 2007). As the mycelium produces and releases oxalic acid, the acid comes into contact with iron ions and chelates the iron. Therefore, as the oxalic acid chelates the iron, it is unable to degrade pectic substances and pathogenicity may be prevented.

A significant difference in the radius of mycelial growth between trials may be attributed to colony age at transfer. While the same isolate was used for both trials, the age of culture varied from one week for the first trial to three days for the second trial. Three day-old cultures were actively growing while the week old cultures were vegetatively mature and grew slower. Wu et al. (2008) showed that mature colonies were slower to resume active growth. Despite variations in growth rate, similar conclusions were drawn from each trial.

## Conclusion

The results from this trial indicated that 5.4 pH is an optimal pH for dollar spot growth. Low (10 to 100 mg kg$^{-1}$) iron concentrations may increase *S. homoeocarpa* mycelial growth. The increase in mycelial growth indicates that *S. homoeocarpa* requires small amounts of available iron for growth and may increase potential pathogenicity. In these trials, increasing the iron concentrations to 1000 mg kg$^{-1}$ suppressed or reduced the potential for *S. homoeocarpa* infection. The bi-weekly application of ferrous sulfate (48.8 kg ha$^{-1}$) used in this trial reduced *S. homoeocarpa* infection of creeping bentgrass and increased turf quality, even during heat stress and high disease pressure. Because of the reduction in *S. homoeocarpa* infection, fungicides used in conjunction with ferrous sulfate may extend the period between fungicide applications and reduce the potential for development of resistant populations.

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# Appendix: Tables and Figures

**Table 1.** Nitrogen fertilizer and fungicide application dates during the recovery periods and the bi-weekly nitrogen application dates.

| Date | Fungicide/Fertilizer [w] | Rate: N or a.i. (kg ha$^{-1}$) | |
|---|---|---|---|
| 3/29 | Urea | 7.3 | Cycle 1 |
| 4/12 | Urea | 7.3 | |
| 4/26 | Urea | 7.3 | |
| 5/10 | Chlorothalonil[x] | 12.5 | |
| | Urea | 7.3 | |
| 5/17 | Urea | 7.3 | Cycle 2 |
| 5/24 | Chlorothalonil | 12.5 | |
| | Urea | 7.3 | |
| 5/30 | Propiconazole[x] | 0.5 | |
| 5/31 | Urea | 7.3 | |
| 6/7 | Chlorothalonil [y] | 16.0 | |
| | Fluoxastrobin | 1.0 | |
| | Urea | 7.3 | |
| 6/21 | Urea | 7.3 | |
| 6/26 | Chlorothalonil | 12.5 | |
| 6/28 | Urea | 7.3 | Cycle 3 |
| 7/5 | Urea | 7.3 | |
| 7/9 | Chlorothalonil | 12.5 | |
| 7/12 | Urea | 7.3 | |
| 7/17 | Chlorothalonil | 8.2 | |
| 7/19 | Urea | 7.3 | |
| 7/26 | Chlorothalonil | 8.2 | |
| | Urea | 7.3 | |
| 8/2 | Chlorothalonil | 8.2 | |
| | Urea | 7.3 | |
| 8/9 | Chlorothalonil | 9.4 | |
| 8/16 | Urea | 7.3 | Cycle 4 |
| 8/30 | Urea | 7.3 | |
| 9/6 | Vinclozolin[z] | 1.51 | |
| | Urea | 7.3 | |
| 9/13 | Urea | 7.3 | End of season recovery |
| 9/20 | Urea | 7.3 | |
| 9/27 | Urea | 7.3 | |

[w] Urea is applied as a liquid using a walk behind sprayer.
[x] Daconil Zn® Sygenta Professional Products, Greensboro, NC
[y] Disarm C® Arysta Life Science Corporation, Cary, NC
[z] Curlan EG® Cleary Chemical Company, Dayton, NJ

**Table 2.** Turf quality and *Sclerotinia homoeocarpa* infection as affected by sulfur (10.3 kg ha$^{-1}$), ferrous sulfate (48.8 kg ha$^{-1}$), and Fe-EDTA at (11.2 kg ha$^{-1}$) fertilizer treatments on a creeping bentgrass putting green in 2012.

| ANOVA | | | |
|---|---|---|---|
| Source | Df | Quality | Dollar spot count |
| Fertilizer | 3 | ** | ** |
| Cycle | 3 | ** | ** |
| Fertilizer x Cycle | 9 | ** | ** |

** = significant at $p < 0.01$.

**Table 3.** Turf quality and *Sclerotinia homoeocarpa* infection center counts as affected by fertilizer treatments and disease cycle on a 'Penn A-4' creeping bentgrass putting green in 2012.

| | Infection centers per plot [w] | | | | Quality [x] | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment and level [v] | Cycle 1 [y] May 10 | Cycle 2 Jun. 27 | Cycle 3 Aug. 9 | Cycle 4 Sep. 4 | Cycle 1 Mar. 28 to May 10 | Cycle 2 May 11 to Jun. 27 | Cycle 3 Jun. 28 to Aug. 9 | Cycle 4 Aug. 10 to Sep. 4 |
| Untreated | 31.0a[z] | 60.6a | 9.4b | 73.6a | 6.5a | 6.4b | 6.8b | 6.7b |
| Sulfur (10.3 kg $\text{ha}^{-1}$) | 22.4a | 53.0a | 31.4a | 84.4a | 6.8a | 6.7b | 6.8b | 6.3b |
| Ferrous Sulfate (48.8 kg $\text{ha}^{-1}$) | 25.4a | 20.0b | 0.4c | 2.4b | 6.5a | 8.1a | 7.8a | 7.8a |
| Fe-EDTA (11.2 at kg $\text{ha}^{-1}$) | 14.8a | 3.0b | 0.0c | 1.0b | 6.7a | 8.3a | 7.2ab | 5.2c |

[v] Fertilizer treatments were applied bi-weekly as liquid applications from March through mid-September.

[w] *Sclerotinia homoeocarpa* infection center counts were completed when control plots averaged 3% surface area infection or when approximately 25 to 30 infection centers were observed.

[x] Quality ratings based on 1-9 scale, where 1 = dead, brown turf and 9 = dense, dark green turf. 6 = minimum acceptable level a putting green. Quality ratings were averaged together for each cycle.

[y] A cycle includes a recovery period, *Sclerotinia homoeocarpa* infection allowed and counted. During the recovery period fungicides are applied and urea (7.3 kg $\text{ha}^{-1}$) was applied weekly instead of bi-weekly to allow the *Sclerotinia homoeocarpa* scars to heal.

[z] Means in the same column followed by the same lower case letter according to Fisher's protected LSD test (P = 0.05).

**Table 4.** The effect of agar pH (4.5, 5.0, 5.5, 6.5) and iron concentration (0, 10, 100, 1000) on *in vitro* radial growth of *Sclerotinia homoeocarpa*.

| ANOVA | | |
|---|---|---|
| Source | Df | Mycelial Growth |
| Trial | 1 | ** |
| pH | 3 | NS |
| Iron | 3 | ** |
| pH x Iron | 9 | NS |
| Trial x pH | 3 | ** |
| Trial x Iron | 3 | ** |
| Trial x pH x Iron | 9 | ** |

** = significant at $p < 0.01$. NS = Not Significant.

**Figure 1.** Interaction of agar pH and iron concentration on *in vitro* radial growth of *Sclerotinia homoeocarpa* in trial 1.

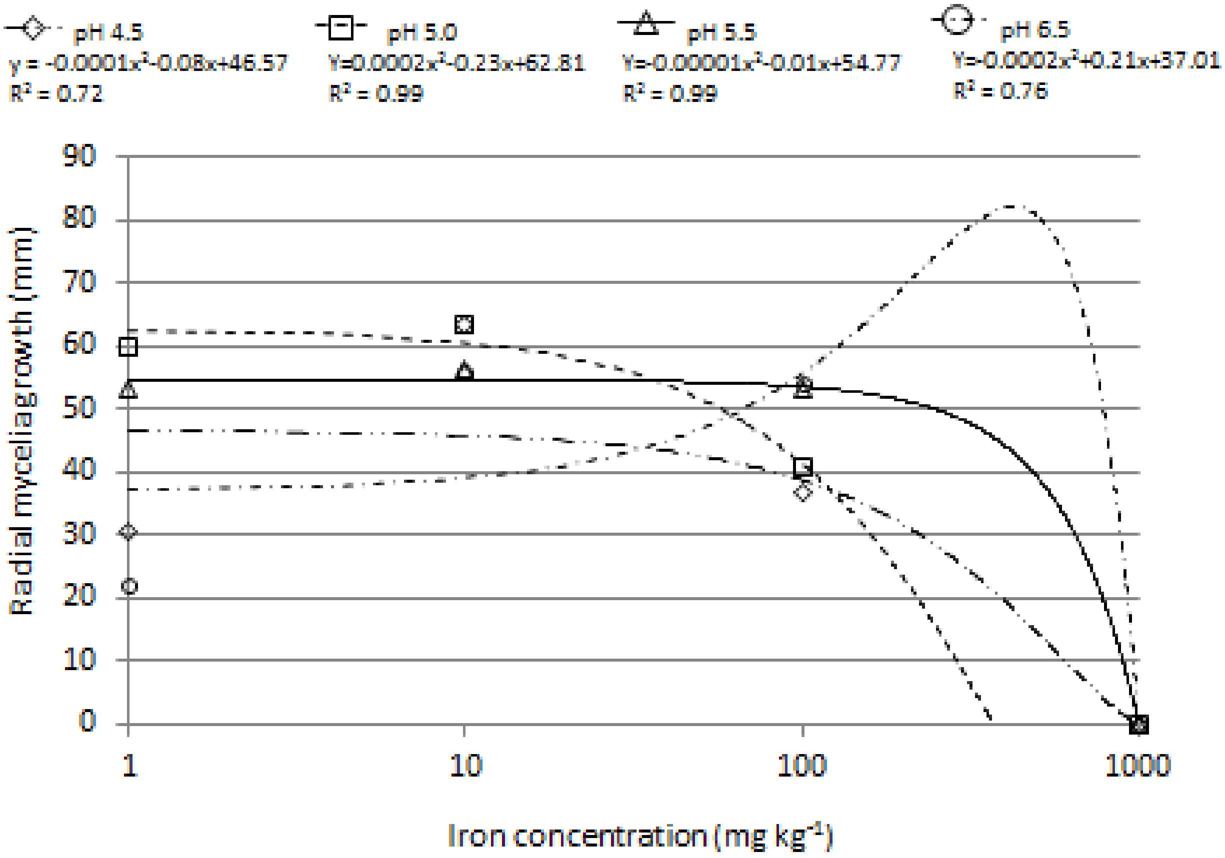

**Figure 2.** Interaction of agar pH and iron concentration on *in vitro* radial growth of *Sclerotinia homoeocarpa* in trial 2.

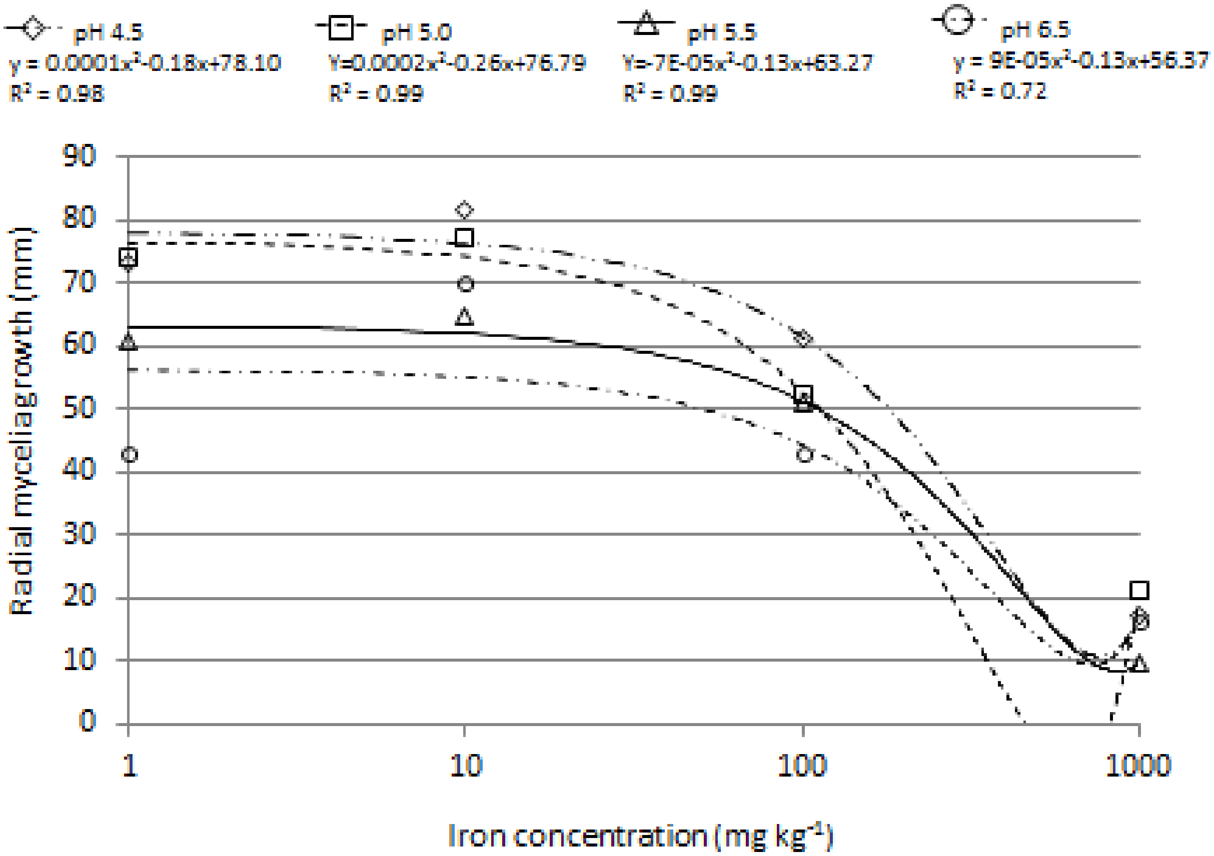

**Figure 3.** Interaction of agar pH and iron concentration on *in vitro* radial growth of *Sclerotinia homoeocarpa* in trial 1.

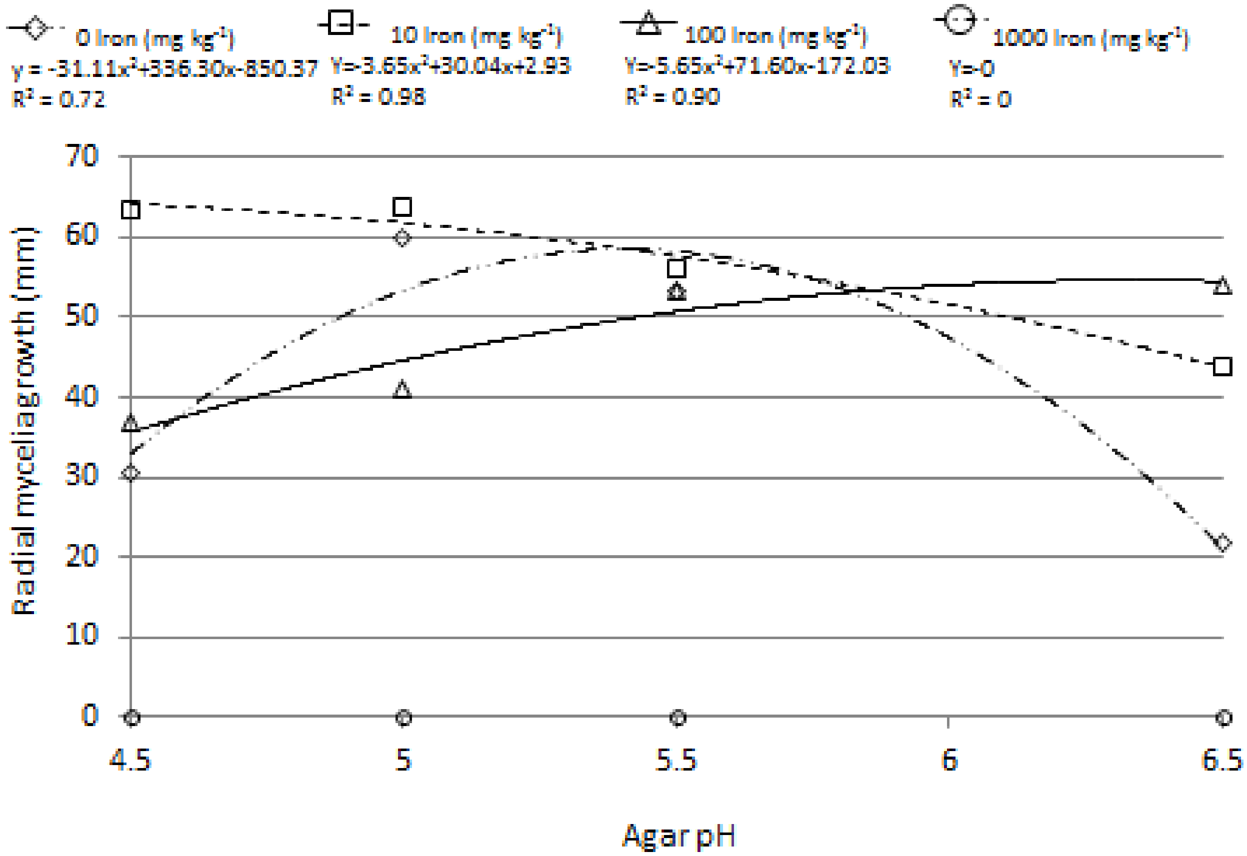

**Figure 4.** Interaction of agar pH and iron concentration on *in vitro* radial growth of *Sclerotinia homoeocarpa* in trial 2.

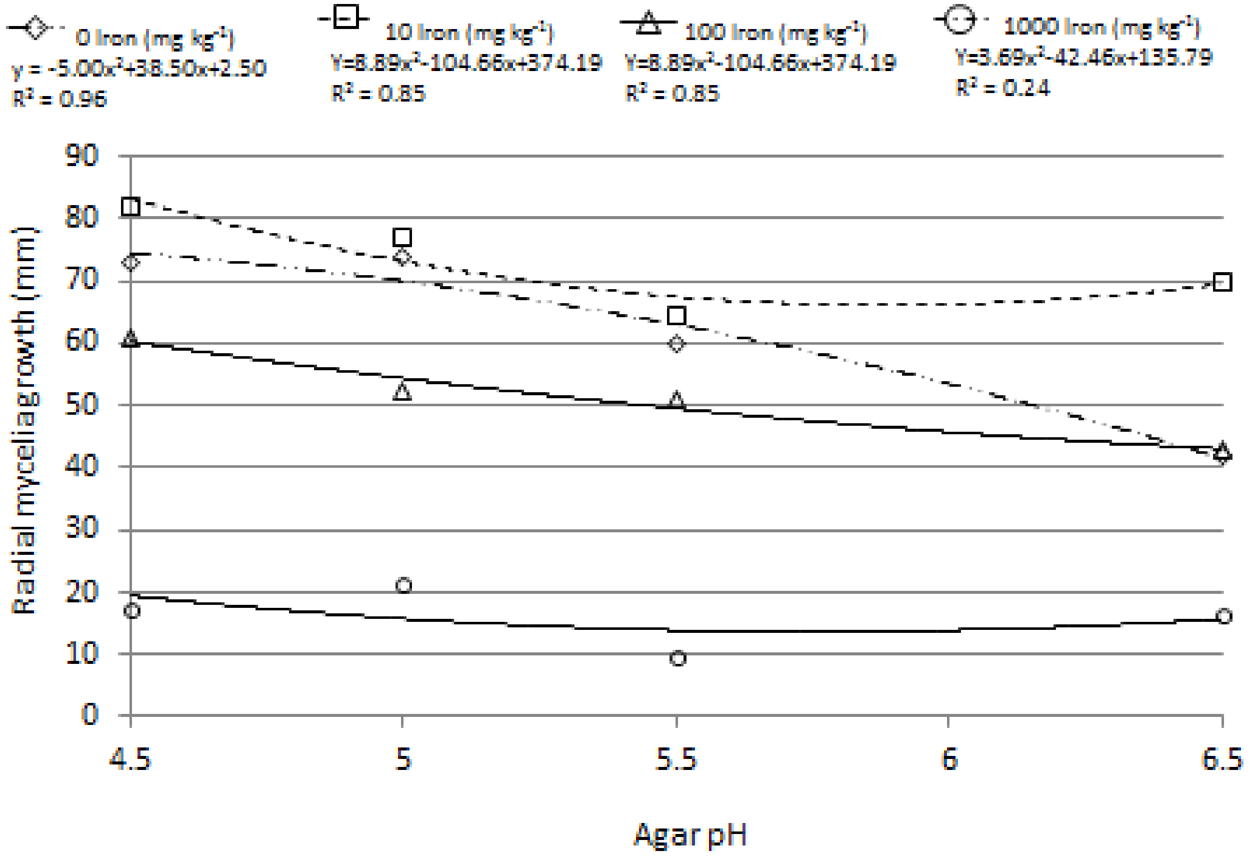

**Figure 5.** Late-summer turf quality as affected by the bi-weekly application of Fe-EDTA (11.2 kg ha$^{-1}$) on creeping bentgrass.

**Figure 6.** *Sclerotinia homoeocarpa* infection as affected by sulfur, ferrous sulfate, and Fe-EDTA fertilizer treatments on a creeping bentgrass putting green in June 2012.

**Figure 7.** Interaction of agar pH and iron concentration on *in vitro* radial growth of *Sclerotinia homoeocarpa* in trial 2.