

CULTURAL AND CHEMICAL CONTROL OF SILVERY-THREAD MOSS IN CREEPING  
BENTGRASS PUTTING GREENS

by

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B.S., Rutgers University, 2009  
M.S., Kansas State University, 2012

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Horticulture, Forestry, and Recreational Resources  
College of Agriculture

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## Abstract

Controlling and preventing the spread of silvery-thread moss (STM, *Bryum argenteum* Hedw.) in putting greens is a difficult task for superintendents. Once established, a STM infestation can quickly increase through the movement of asexual propagules, such as shoot fragments, bulbils and protonema. Many practices used by superintendents are likely aiding in the dispersal and establishment of propagules. Research is needed to help superintendents effectively control this very invasive pest. The objectives of this research were to: 1) Investigate the cumulative effect of cultivation on a STM infestation in a creeping bentgrass putting green, when used with or without light, frequent topdressing and the herbicide carfentrazone; 2) Determine if STM growth is reduced by spraying ammonium sulfate (AMS) compared to urea, and understand the effect of spray volume on STM growth; 3) Evaluate the effectiveness of a range of carfentrazone rates for postemergence STM control; and 4) Determine if altering the pH of irrigation water with sulfuric- or hydrochloric acid affects the growth of STM. Generally, cultivation and carfentrazone reduced STM cover; however, the greatest reduction in STM cover was achieved when cultivation treatments were used in conjunction with carfentrazone. Topdressing did not affect STM cover. Ammonium sulfate increased STM cover and dry weight compared to urea and an untreated control. Furthermore, spray volume did not affect STM cover at any rating date. Superintendents managing STM infestations should limit or avoid use of AMS as an N source. At 28 days after treatment (DAT) the ED<sub>90</sub> (dose required to cause 90% gametophyte injury) was 26.8 g ai ha<sup>-1</sup>, and at 49 DAT ED<sub>90</sub> was 54.3 g ai ha<sup>-1</sup>; both of these doses are substantially lower than the label rates for long- and short-term control, respectively. As compared with label recommendations, this research suggests lower carfentrazone rates, and longer intervals, may be effective for STM control in putting greens. Irrigation pH affected STM

growth, with pH's 5 and 6 having increased growth compared to pH's 7 and 8. It was hypothesized a lower irrigation pH enabled STM to better withstand sodium stress.

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## **Dedication**

I dedicate this work to my wonderful wife and daughter. None of those this would have been possible without the both of you by my side. I am excited to see what the future holds for the Raudenbush's!

# **Chapter 1 - A Review: Establishment, dispersal, and management of silvery-thread moss (*Bryum argenteum* Hedw.) in creeping bentgrass putting greens**

## **Introduction**

Controlling and preventing the spread of silvery-thread moss (*Bryum argenteum* Hedw.) in golf course putting greens is a difficult task for golf course superintendents. *Bryum argenteum* is undesirable because it can negatively affect ball roll and surface uniformity. The prevalence of *B. argenteum* in golf course putting greens has received attention from several researchers in recent decades (Boesch and Mitkowski, 2005; Borst et al., 2010; Kennelly et al., 2010; Thompson et al., 2011). Although no published research has directly established a connection between mercury-based fungicides and the prevalence of *B. argenteum* on putting greens, some researchers speculate the discontinued use of mercury-based fungicides plays an important role (Boesch and Mitkowski, 2005; Borst et al., 2010; Burnell et al., 2004; Happ, 1998; Rossi, 2002; Yelverton, 2005) because bryophytes are relatively intolerant of heavy metals (Weber and McAvoy, 2003). Additionally, in recent decades there have been improvements in equipment technology, nutrient management, putting green construction, and changes in golfer expectations that have influenced the intensity and scope of cultural practices utilized by golf course superintendents. It is likely several of these cultural practices are impacting the establishment and infestation of *B. argenteum* (Kennelly et al., 2010; Thompson et al., 2011). Furthermore, a thorough understanding of a target weed's biology, morphology, and physiology is essential to formulating hypotheses for testing best management practices for effective weed control

(Radosevich et al., 2007; Sutherland, 2004). Therefore, the objective of this literature review is to discuss relevant biological and ecological traits of *B. argenteum* and how they potentially interact with current turfgrass management practices utilized by golf course superintendents. With this information, researchers and practitioners will be better equipped to develop successful long-term management strategies for *B. argenteum* in golf course putting greens.

### **Morphology, reproduction, and dispersal**

The morphological and reproductive characteristics of *B. argenteum* uniquely affect its behavior in putting greens; thus, a thorough understanding of these traits is necessary to develop successful control strategies. *Bryum argenteum* is a member of the Bryopsida class of bryophytes. Bryopsida contains 84% of all bryophyte families and is by far the most diverse class (Glime, 2007). *Bryum argenteum* is a cosmopolitan species found on every continent (Crum and Anderson, 1981). It is highly polymorphic, with several different ecotypes and growth rates experimentally demonstrated (Longton 1981; Shaw and Albright, 1990; Horsley et al., 2011). The upper leaves of *B. argenteum* have a layer of clear cells, giving it a silvery sheen, and hence the common name silvery-thread moss (Crum and Anderson, 1981).

An established *B. argenteum* colony is comprised of individual gametophores, protonema, and rhizoids (Figures 1.1 & 1.2). The stems and leaves of bryophytes are known as gametophores or shoots, which grow from an apical meristem containing a prominent apical cell (Mauseth, 2003). Gametophores can form from structures produced during sexual or asexual reproduction. Completion of the sexual cycle produces a diploid sporophyte containing genes from both male and female parents (Figure 1.3). The distal region of the sporophyte expands and undergoes meiosis, producing thousands of haploid spores. During favorable conditions the

spore germinates, giving rise to chlorophyllous cells that undergo mitosis to produce a mass of chloroplast-containing thread-like green filaments called primary protonema (Vanderpoorten and Goffinet, 2009). Interestingly, *B. argenteum* can also produce secondary protonema from fragments, bulbils or rhizoidal tubers (Frey and Kürschner, 2011). Protonema are capable of growing along the surface of almost any stable structure, but can easily desiccate if moisture is not available (Proctor et al., 2007). As protonema develop, buds are produced giving rise to an individual shoot (the gametophore), which differentiates into stems and leaves. A mass of gametophores (shoots) is known as the gametophyte. In laboratory studies where *B. argenteum* was cultured from individual bulbils, Horsley et al. (2011) reported approximately 11 days were required until the emergence of protonema, and 31 days until the production of the first gametophore. Rhizoids are produced from the underside of the protonema or directly from bulbils and gametophore fragments (Vanderpoorten and Goffinet, 2009). *Bryum argenteum* produces an extensive rhizoid system, enabling it to anchor to almost any substrate. This is similar to the roots of vascular plants, but *B. argenteum* rhizoids do not appear to have the ability to conduct water and nutrients internally (Glime, 2007).

Other characteristics of *B. argenteum* include dioecy and the capability of both sexual and asexual reproduction (Crum and Anderson, 1981); however, the most advantageous mode of reproduction is not clear. Sexual reproductive structures form on the tips of separate gametophores; female organs are known as the archegonia and male organs are antheridia (Vanderpoorten and Goffinet, 2009). In the presence of water, biflagellate sperm are released from the antheridia and swim to fertilize the archegonia, producing a diploid sporophyte (Figure 1.3). The sporophyte grows as an embryo via mitosis until emerging and producing an elongated seta (stalk). The distal region of the seta undergoes meiosis to produce haploid spores, restarting

the cycle (Vanderpoorten and Goffinet, 2009). Spores can be carried long distances by water, wind and machinery. Longton and Miles (1982) reported *B. argenteum* colonies are capable of producing 3.7 million spores m<sup>-2</sup> annually; thus, spores serve as the primary mechanism for long distance dispersal (Selkirk et al., 1998). Crum (1972) reported that spores 8 to 12 µm in diameter are capable of traveling over 19,000 km by wind. However, the role of spores in the initial invasion and infestation of putting greens is unclear. Putting greens are typically mown daily, at cutting heights < 3.4 mm and clippings are collected. It is likely the seta stalks (Figure 1.3, part f) are removed via mowing or simply crushed by the weight of the mower; therefore, spore production is unlikely to be a viable mode of reproduction for established *B. argenteum* in a putting green. However, spores may play a role in the initial stages of invasion in a putting green that was previously uninhabited by *B. argenteum*. Sexual reproduction produces genetically unique individuals, which may provide an ecological advantage in a changing environment. Despite this capacity for sexual reproduction, Longton and Miles (1982) monitored 690 moss species found in England, and only 50% regularly formed sporophytes, with 18% never forming sporophytes. Therefore, bryophytes arguably rely on asexual reproduction for colonizing new habitats more than any other plant group (Frey and Kürschner, 2011).

Once established, *B. argenteum* can spread asexually, serving as the primary mechanism for further infestation (Selkirk et al., 1998). Regeneration can occur from cloning (fragmentation) or through the production of specialized caducous organs, such as bulbils or shoot apices (Frey and Kürschner, 2011). Fragmentation is a simple form of vegetative reproduction in bryophytes and occurs when a piece of gametophore is separated from the gametophyte (Frey and Kürschner, 2010). Fragments are capable of traveling long distances, but are typically deposited in close proximity to the original gametophyte. Fragments contain all the

genetic information necessary to establish a new plant. Once deposited in a favorable site, secondary protonema radiate from the fragment (Figure 1.4), producing several hundred new shoots (Frey and Kürschner, 2010). Additionally, the fragments can grow directly into a whole plant with rhizoids developing from their basal parts (Imura and Iwatsuki, 1990).

When no sporophytes are present, *B. argenteum* stems can actively produce a large amount of deciduous shoot apices called bulbils (Selkirk et al., 1998; Stark et al., 2010). Bulbils are small, highly condensed leaf primordia surrounding shoot apical meristems that occur in the axillary position (Figure 1.3); one to several can be produced per shoot (Frey and Kürschner, 2011). Once transported to a favorable site, bulbils “germinate”. A germinating bulbil can produce a shoot directly or give rise to secondary protonema (Frey and Kürschner, 2011). In laboratory studies, Horsley et al. (2011) found bulbil production to be similar between populations of *B. argenteum* collected from Arizona, New Mexico, and Kentucky; moreover, only sixty days were required for a single shoot to produce ten bulbils. It quickly becomes apparent how important bulbil production is in the spread of *B. argenteum*, considering a 5 cm diam. gametophyte contains thousands of shoots, each capable of producing ten bulbils in only sixty days.

Gametophore fragments and bulbils are buoyant and readily transported by water; therefore, a heavy rainfall or irrigation event can move vegetative structures to previously uninhabited areas (Proctor et al., 2007). Additionally, propagules (bulbils, shoot fragments, and protonemal fragments) can be transported from green to green by adhering to golfers’ shoes and to maintenance equipment. So, while sexual reproduction is important for long distance dispersal of spores to previously uninhabited areas, researchers have suggested that movement of vegetative structures serves as the primary mode of short distance dispersal (Frey and Kürschner,

2011; Selkirk et al., 1998); consequently, the effect of putting green management practices on the production, dispersal, and establishment of asexual propagules is of extreme importance.

### **Water relations**

The management regime for a putting green is dictated by a variety of factors, such as turfgrass species, soil rootzone properties, operating budget, and golfer expectations. These differences make it difficult to pinpoint specific causes for increased incidence of *B. argenteum*. The current trend in putting green management is “firm and fast”. Such conditions are obtained through several avenues, but an important practice is reducing the quantity of irrigation, while increasing frequency to produce a firm putting surface with faster ball roll speed (Brame, 2008). *Bryum argenteum* is non-vascular and therefore obtains minimal water and nutrients from the soil (Glime, 2007). In addition, *B. argenteum* is poikilohydric, meaning the water content of the gametophyte will tend to equilibrate with the water status of its surrounding environment (Green et al., 2011). However, this does not mean its cellular water content is constantly fluctuating with changes in the environment because there are several morphological traits *B. argenteum* possesses to deal with water losses (Proctor et al., 2007). For instance, during periods of drought, *B. argenteum* will completely dehydrate and enter a dormant state, possibly for several years, and growth will reinitiate when water is no longer limiting (Happ, 1998). Very little decomposition of *B. argenteum* may occur during prolonged periods of dormancy, because bryophytes generally have a slower decomposition rate compared to vascular plants (Scheffer et al., 2001), and liquid water is required for decomposition. Many bryophytes resist decay by producing secondary compounds, including phenolics, which can inhibit the organisms that

typically facilitate decomposition (Glime, 2007) and is considered a critical attribute for a plant needing to survive prolonged dormancy.

*Bryum argenteum* is poikilohydric (Stark et al., 2010); therefore, irrigation practices have a major effect on its competitiveness and establishment (Lyons et al., 2012). Putting green rootzones are often comprised primarily of sand because most native soils do not possess the physical properties necessary to withstand compaction from frequent traffic (Kunze, 1956). Furthermore, sand is highly porous relative to most native soils. The rootzone mix, outlined in the United States Golf Association's "Green Section Recommendations for a Method of Putting Green Construction", is composed primarily of sand and requires a minimum saturated hydraulic conductivity of 152.4 mm/hr (United States Golf Association Green Section, 2004). Therefore, superintendents often irrigate on a daily basis, especially in summer months when turfgrass roots recede and evapotranspiration rates are high. This practice undoubtedly has a significant impact on moss establishment in putting greens. In a greenhouse experiment, Lyons et al. (2012) irrigated pots containing ground *B. argenteum* shoots with either 75% or 100%  $E_o$  (open pan evaporation) at four watering frequencies (1-, 2-, 4-, and 7-day intervals). Results indicated no differences in the number of *B. argenteum* shoots produced between the irrigation levels; however, daily watering resulted in higher shoot counts compared to pots watered on every fourth or seventh day. This research suggests the higher irrigation frequency necessary to maintain putting greens favors the establishment of individual *B. argenteum* propagules.

In addition to being poikilohydric, *B. argenteum* is also ectohydric, meaning water is transported primarily along the external surfaces of the leaf through capillary action (Glime, 2007; Jones and Rosentreter, 2006). Unlike vascular plants, *B. argenteum* lacks the specialized vascular tissue necessary for rapid internal water movement; therefore, it relies mainly on

capillary action along external leaf and stem surfaces for rapid transport (Glime, 2007). Internal water movement is a rather slow process compared to external capillary movement. Because the dense mat of shoots creates a large amount of surface tension, *B. argenteum* can store and regulate water, while minimizing losses of water to gas exchange (Proctor, 1979). By contrast, isolated shoots do not have the luxury of high surface tension and dry out much more quickly compared to an established gametophyte (Proctor et al., 2007). In the presence of extracellular water, *B. argenteum* remains active but cells will quickly dehydrate and cease metabolism when extracellular water has been exhausted (Proctor et al., 2007). Therefore, *B. argenteum* typically remains in one of two states, fully turgid or desiccated (Proctor et al., 2007). *Bryum argenteum* is usually fully turgid in well-watered putting greens (personal observation).

No published research has identified a connection between poorly drained putting greens and the establishment of silvery-thread moss; however, a moist surface is likely to enhance the survival of dispersed propagules (fragments, bulbils, protonema) and established gametophytes. Superintendents struggling with *B. argenteum* infestations should examine the putting green soil profile to determine if excessive thatch or layering are reducing infiltration rates. If layering exists, then hollow tine aerification should be considered. For instance, over a two year period, McCarty et al. (2007) aerified with hollow tines on eight separate occasions and reported a 150% increase in water infiltration rates on average for aerified plots compared to untreated plots at the conclusion of the study.

In a frequently watered putting green *B. argenteum* has some distinct advantages compared to turfgrass plants. For instance, the dense mat of shoots in a gametophytic colony is often fully immersed in the laminar boundary layer created by the surrounding turfgrass plants (Proctor et al., 2007), reducing water loss. In addition, high surface tension created by the dense

mat of shoots allows *B. argenteum* to retain extracellular water from frequent irrigation or dew (Glime, 2007), compared to turfgrass which is completely reliant on adequate soil moisture within the rootzone profile. Lastly, the poikilohydry of *B. argenteum* enables it to desiccate when extracellular water is not present and quickly reinitiate growth when water is available (Proctor et al., 2007). To maintain a firm putting surface, many superintendents reduce the quantity at each irrigation event, but increase the frequency; therefore, extracellular water may seldom be limiting, allowing *B. argenteum* to remain in an active, turgid state throughout most of the growing season.

## **Nutrition**

Fertilization is another important component to maintain acceptable turfgrass quality throughout the growing season. For creeping bentgrass growing in sand-based rootzones, agronomists typically recommend applying 16 to 32 kg N ha<sup>-1</sup> every month during the growing season (Dernoeden, 2013). Superintendents typically “spoon-feed” nitrogen throughout the growing season for several reasons: 1) sand-based rootzones inherently have low cation exchange capacity; 2) applying 50 to 100 kg N ha<sup>-1</sup> in a single fertilization event in the summer months can cause a flush of growth, leading to excessive thatch buildup and potentially leaving the canopy prone to scalping and creating environmental conditions favorable for diseases; and 3) many superintendents apply preventative fungicides and plant growth regulators about every 14 days in the summer months, and soluble nitrogen fertilizers such as urea and ammonium sulfate are often included in the spray mixture at low rates ( $\leq 10$  kg N ha<sup>-1</sup>).

This spoon-feeding N approach likely favors *B. argenteum*. Bryophytes have about one-tenth the nutrient requirement of higher plants, with excessive nutrient amounts potentially being

harmful (Glime, 2007). In liverworts, Voth (1943) applied an array of nutrient concentrations to *Marchantia polymorpha* and reported decreased dry weight and thallus growth as nutrient concentrations increased. By contrast, Thompson et al. (2011) sprayed urea at 15 kg N ha<sup>-1</sup> biweekly throughout the growing season and reported a 147% to 155% increase in *B. argenteum* infestation. Furthermore, Jones and Rosentreter (2006) reported *B. argenteum* growth in a sand substrate was greatest when fertilized with a solution containing macro and micronutrients, compared to macronutrients alone. These results suggest that *B. argenteum* can readily use nutrients when available, despite minimal nutrient requirements of most bryophytes.

### **Mowing and other cultural practices**

The factors primarily responsible for the dispersal of asexual *B. argenteum* propagules are currently unknown. Researchers have attributed the increased incidence of *B. argenteum* in putting greens to excessively low cutting heights (Kennelly et al., 2010). In Kansas, greater moss cover in a creeping bentgrass putting green was reported when plots were mown at 3.2 mm compared to plots mown at 4.0 mm (Kennelly et al., 2010). Lower cutting heights can lead to stressed turfgrass plants, decreasing their competitiveness against weed species. Furthermore, the likelihood of the mower removing or dislodging individual gametophores increases as cutting heights decrease; such gametophore fragments may then be dispersed around the putting green on equipment or golfers' shoes. Mower contact with gametophytes may also be increased where frequent nutrient applications result in the growth of longer gametophores.

By contrast, topdressing greens is a management practice that may reduce *B. argenteum*. Frequent topdressing applies a thin layer of sand to the turfgrass canopy every 2 to 4 weeks at a typical rate of 1200 to 2400 kg sand ha<sup>-1</sup> (Dernoeden, 2013). Turfgrass plants quickly grow

through the layer of sand, but the vertical growth rate of *B. argenteum* is much slower in relation to turfgrass. Borst et al. (2010) reported four biweekly topdressing applications reduced *B. argenteum* cover by 34% in a creeping bentgrass putting green. This reduction may have occurred because topdressing elevated the effective height of cut in relation to the gametophyte, reducing the incidence of fragmentation caused by mowing, compared to a green that does not receive frequent topdressing. Furthermore, topdressing dilutes thatch, providing a firmer surface for the mower to ride upon (Dernoeden, 2013). If thatch is excessive, the mower will sink into the turf, lowering the effective height of cut and increasing the likelihood of the mower clipping *B. argenteum* gametophytes. Therefore, strategies aimed at reducing the removal of gametophore tips during mowing may reduce the amount of plant material available for dispersal, and possibly delay the infestation of *B. argenteum*. Many of the cultural practices utilized by superintendents such as, aerification, grooming, verticutting, and brushing are aimed at manipulating the turfgrass canopy and are likely affecting the fragmentation and dispersal of *B. argenteum*. Currently, these practices have not been evaluated for their effects on *B. argenteum* establishment, but this information would be helpful to superintendents attempting to manage existing infestations.

### **Chemical management**

Several researchers have conducted studies and reviews on the current chemical control strategies for reducing *B. argenteum* in putting greens (Boesch and Mitkowski, 2005; Borst et al., 2010; Burnell et al., 2004; Kennelly et al., 2010; Thompson et al., 2011). All achieved some level of success, but none were effective at completely eradicating *B. argenteum* from putting greens. Arguably, the most effective control has been achieved with the herbicide carfentrazone-

ethyl, but control has been inconsistent and temporary (Boesch and Mitkowski, 2005; Borst et al., 2010; Kennelly et al., 2010; Thompson et al., 2011).

Carfentrazone-ethyl is a protoporphyrinogen oxidase (PPO) inhibitor that causes rapid necrosis via lipid peroxidation (Senseman, 2007). Active photosynthesis is required to cause necrosis because PPO catalyzes the oxidation of protoporphyrinogen IX, which is a light dependent process (Senseman, 2007). Active photosynthesis in *B. argenteum* occurs in the green tissue located at the upper portion of the gametophore; however, regeneration can occur from lower segments on the gametophore where photosynthesis is not actively occurring. Application of carfentrazone-ethyl causes lipid peroxidation in actively growing gametophore tips, but reactive oxygen species are not likely produced in the lower, non-photosynthetically active portions of the gametophyte. Hence, turfgrass managers should not expect long-term control from a single application of carfentrazone-ethyl. Moreover, because *B. argenteum* is poikilohydric, it has the potential to be in a non-photosynthetically active state if extracellular water is not present, which would reduce the efficacy of a carfentrazone-ethyl application. Research regarding the effects of cellular water content of *B. argenteum* on efficacy of carfentrazone-ethyl would be valuable to superintendents.

### **Multifaceted management approach**

Current management practices on golf course putting greens, designed to provide a firm, fast putting surface, appear to complement the ecology and biology of *B. argenteum*. Water is seldom limiting in many putting greens, allowing *B. argenteum* to remain active throughout most of the growing season. In putting greens where *B. argenteum* is prevalent we recommend superintendents pay strict attention to their water management practices. A higher irrigation

frequency is likely to favor the establishment of deposited bulbils and fragments, leading to further infestation. The presence of extracellular water dictates whether *B. argenteum* is active or dormant, so limiting the number of irrigation events within a given period should encourage the latter state. *Bryum argenteum*'s internal water content is directly related to its surrounding environment; therefore, superintendents should increase air movement through the use of fans or selective removal of surrounding vegetation to encourage desiccation of asexual propagules and gametophytes. Ultimately, a deep and extensive root system can allow turfgrass greater access to water within the profile for longer periods of time, decreasing the need for frequent irrigation events (Jordan et al., 2003). Management practices that encourage root growth in the spring and fall are recommended.

Frequent applications of nitrogen enhance the growth and competitiveness of *B. argenteum* in putting greens; however, reducing fertility is not recommended as a means of control. Improper fertility can lead to increased incidence of several diseases and reduce the healing of ball marks, all of which create available sites for *B. argenteum* propagules to grow. Furthermore, a good fertility program is essential to producing an extensive root system (Schlossberg and Karnok, 2001), which should enable superintendents to decrease irrigation frequency.

Frequent topdressing has been shown to reduce *B. argenteum*. Topdressing provides a firmer surface for the mower to ride upon, decreasing the likelihood of the bedknife or reel coming in contact with the gametophyte, especially at low cutting heights. Topdressing sand is often brushed into the canopy to help reduce the amount of material picked up by the mowers. Research is not available regarding the effects of brooming/brushing on dispersal of *B. argenteum* propagules; however, it is likely to physically dislodge vegetative propagules.

The importance of monitoring for *B. argenteum* cannot be over emphasized because a colony 25 mm in diameter can contain thousands of individual shoots, each capable of establishing a new colony. Additionally, the ability of *B. argenteum* to retain and absorb extracellular water increases with the size of the gametophyte. Individual gametophores deposited away from the original colony are highly susceptible to desiccation because they lack the strong adhesive forces to hold onto extracellular water. Furthermore, the amount of asexual plant material available for dispersal increases drastically as the size of the colony grows. The efficacy of control measures is likely to be enhanced if an invasion of *B. argenteum* is caught early; however, control may be much more difficult during the later stages of an infestation.

### **Conclusion**

The “silver-bullet” for selective *B. argenteum* control does not seem likely in the near future, but management practices aimed at reducing the number of available propagules for dispersal in conjunction with the current control measures (Table 1.1) are likely to keep populations low. Lastly, from personal experience, superintendents typically battle with *B. argenteum* in select greens on the property. This begs the question: Why isn’t it a major problem on every green? Several factors could be to blame, but ultimately, those greens likely contain a microenvironment that is optimal for *B. argenteum*. Superintendents should consider which factors are contributing to the success of *B. argenteum* on infested greens and address them accordingly in order to obtain successful long-term control.

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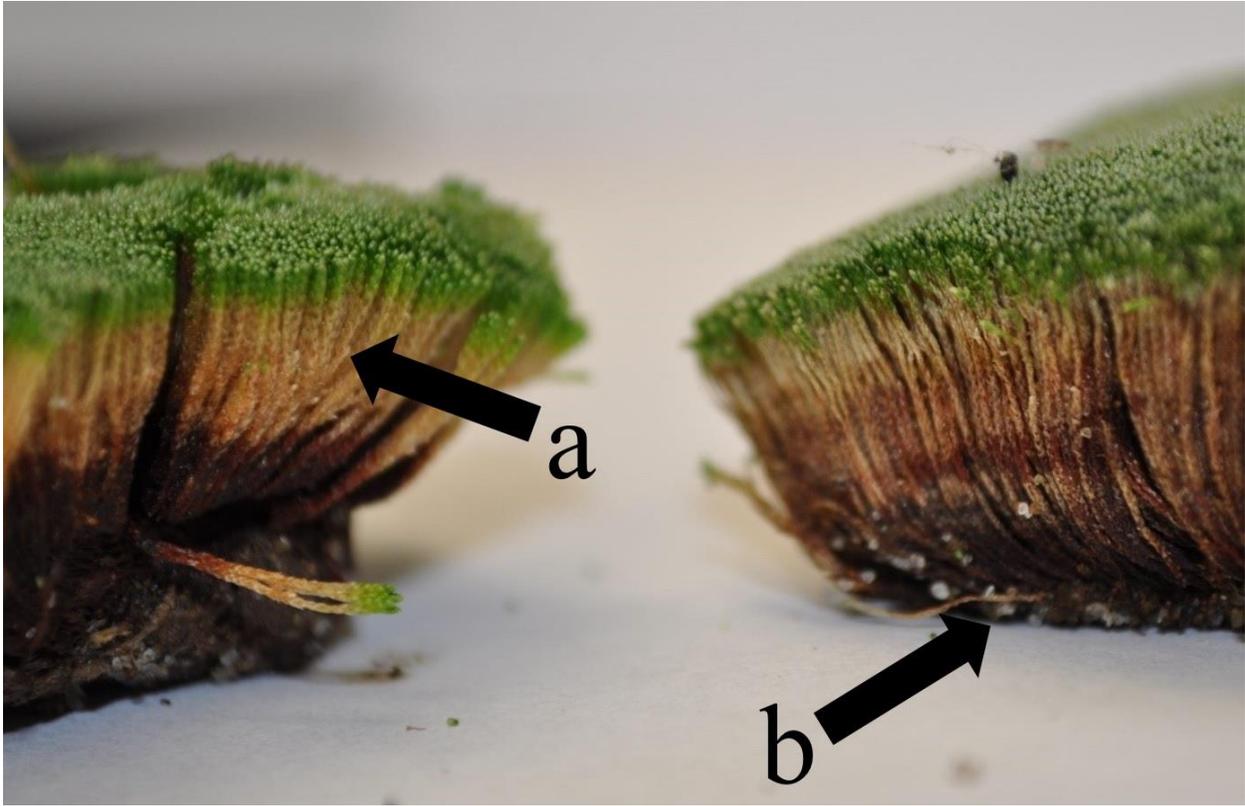


Figure 1.1 *Bryum argenteum* (Hedw.) gametophyte containing a) gametophores and; b) rhizoid mat.



Figure 1.2 Production of *Bryum argenteum* (Hedw.) gametophores from secondary protonema.

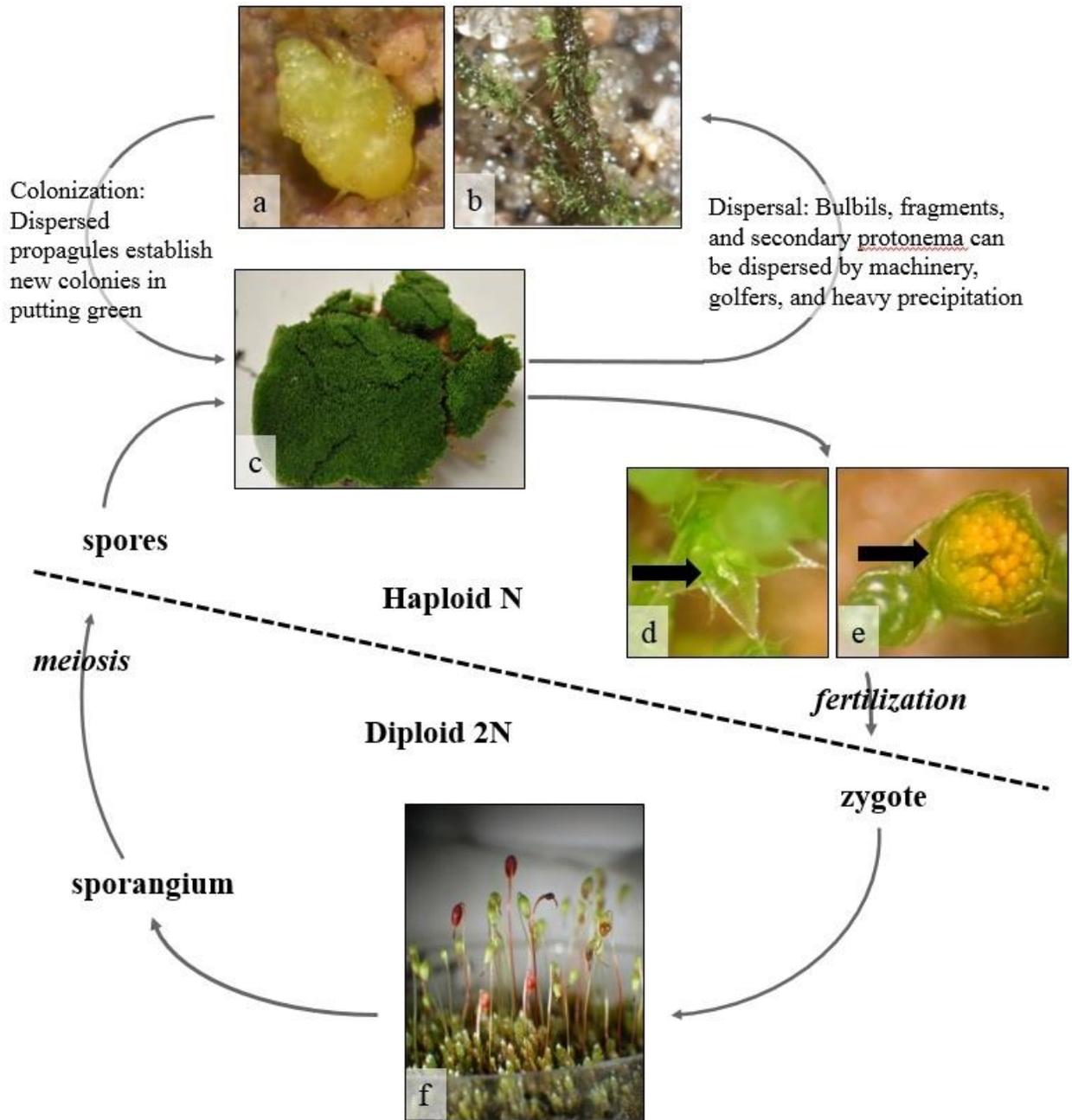


Figure 1.3 Life cycle diagram of silvery-thread moss (*Bryum argenteum* Hedw.) displaying sexual and asexual cycles, ploidy, and dispersal processes: a) bulbil; b) secondary protonema produced from existing gametophore; c) established gametophyte containing individual gametophores; d) female gametophore with arrow pointed at archegonia; e) male

gametophore with arrow pointed at antheridia; f) group of sporophytes raised in a petri dish under laboratory conditions.

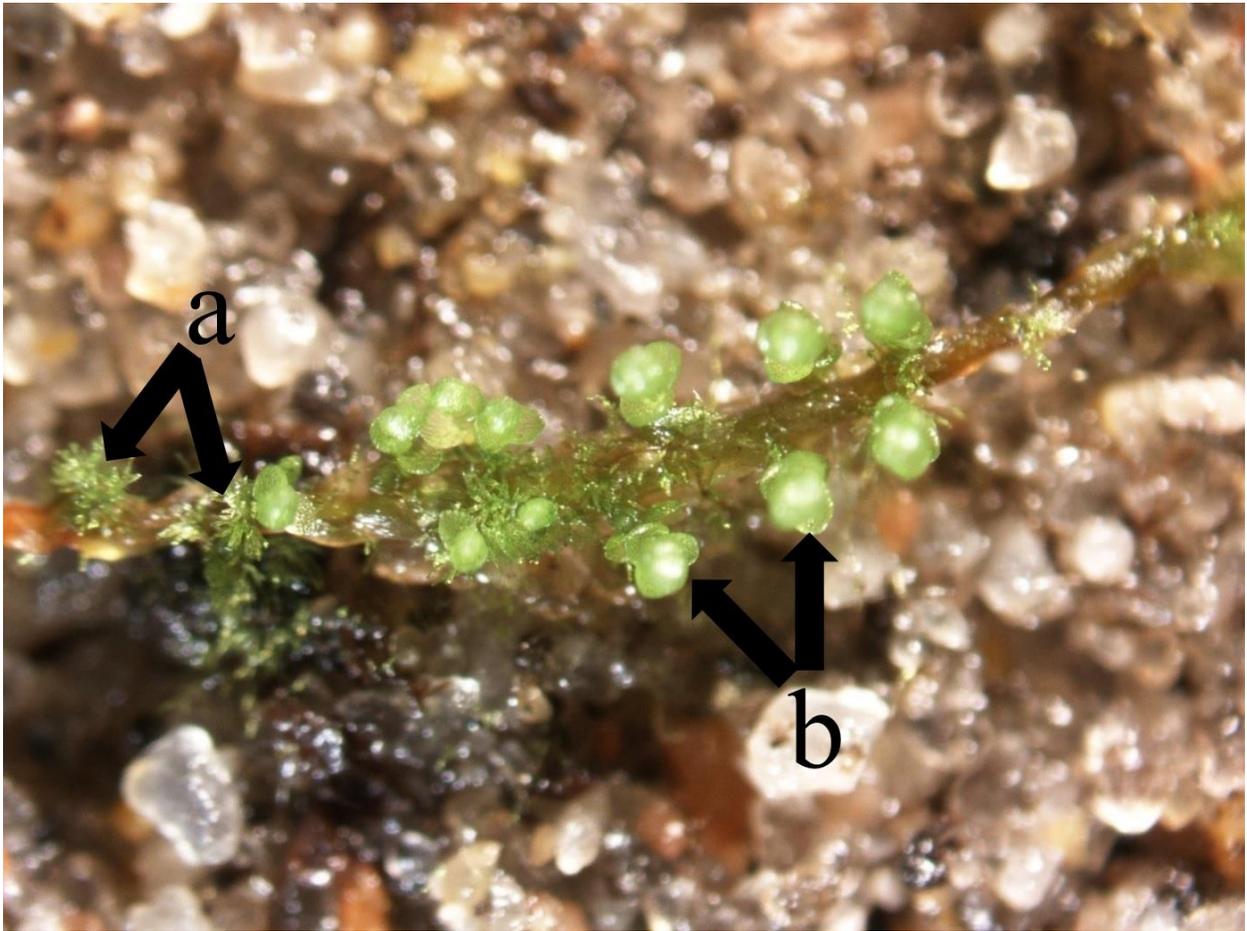


Figure 1.4 Parent shoot of *Bryum argenteum* (Hedw.) treated with carfentrazone-ethyl producing; a) secondary protonema and; b) new leaf primordia one week after treatment.

Table 1.1 Best management practices for silvery-thread moss (*Bryum argenteum* Hedw.) control in golf course putting greens.

- 
- Reduce irrigation frequency to encourage desiccation of disseminated shoot fragments, bulbils, and protonema.
  - Implement a light and frequent topdressing program throughout the growing season.
  - Reduce the frequency of soluble-nitrogen applications; consider substituting organic nitrogen applications in spring and fall.
  - Applications of carfentrazone-ethyl should be used in combination with cultural practices, as the herbicide is effective at reducing the size of infestations, but does not usually lead to complete eradication.
  - Increase air movement via fans or selective removal of surrounding vegetation to encourage light penetration and air circulation.
  - Increase cutting heights.
  - Address factors contributing to poor drainage and infiltration.
-

## **Chapter 2 - Cultivation Reduces Infestation of Silvery-Thread Moss (*Bryum argenteum* Hedw.) in a Creeping Bentgrass (*Agrostis stolonifera* L.) Putting Green**

### **Abstract**

Cultivation creates voids in a putting green that may be recolonized by weeds or creeping bentgrass; therefore, we investigated the cumulative effect of cultivation on a silvery-thread moss (STM) infestation in a creeping bentgrass putting green, when used with or without light, frequent topdressing and the herbicide carfentrazone. Cultivation treatments were applied spring and fall, and included hollow-tine aerification at low- and high surface disruption (SD), vertislicing, and no cultivation. Carfentrazone was applied one week before and after cultivation treatments each spring and fall. On average, each split-application of carfentrazone reduced STM cover by ~20%. Cultivation did not increase STM cover; to the contrary, hollow-tine aerification at low SD and vertislicing slightly reduced STM cover, even in the absence of carfentrazone. Topdressing did not affect STM cover. The greatest reduction in STM cover was achieved when cultivation treatments were used in conjunction with carfentrazone.

### **Introduction**

Controlling silvery-thread moss (STM) infestations in golf course putting greens can be a difficult task because of STM's unique morphological and biological properties. Silvery-thread moss is a perennial species capable of tolerating a wide range of environmental conditions and is highly competitive at low temperatures (Crum and Anderson 1981; Longton 1981), when the

relative growth rate of desirable cool-season turfgrass species is low. An infestation of STM typically begins as small colonies (< 5 cm in diameter), but it can rapidly increase in size if environmental conditions are conducive for its growth and dispersal. Silvery-thread moss can invade new habitats via production of sexual and asexual structures, but once established it is believed to spread exclusively through asexual avenues (Raudenbush et al. 2015; Selkirk et al. 1998). Two common modes of asexual reproduction in STM are fragmentation and bulbil production (Frey and Kurschner 2011; Horsley et al. 2011; Jones and Rosentreter 2006; Selkirk et al. 1998). Fragmentation is the physical movement of an existing STM filament. Filaments can be dislodged by machinery, heavy irrigation/precipitation, or golfers' spikes. Once deposited, the dislodged filaments can grow directly into new shoots, or produce secondary protonema which give rise to new shoots. Bubbles are highly condensed leaf primordia and are actively produced by an existing STM shoot (Frey and Kurschner 2011). Bubbles are buoyant; consequently, they are readily transported during a heavy irrigation or precipitation event. Once deposited, bubbles may produce secondary protonema or new shoots. Horsley et al. (2011) collected several STM populations and reported individual shoots were capable of producing 10 bubbles in only sixty days. All of these factors make it imperative that golf course superintendents take action early in a STM infestation. Left unchecked, a small infestation can quickly spread throughout a putting green due to the massive amount of propagule material and its potential to proliferate.

Superintendents utilize an array of tools and cultural practices, and these tools often affect the population dynamics of STM in a creeping bentgrass putting green. For example, Kennelly et al. (2010) reported a cutting height of 3.2 mm resulted in a 1.6-fold increase in STM severity compared to 4.0 mm. Thompson et al. (2012a) investigated different N sources and discovered soluble N sources increased STM severity in a putting green. Irrigation practices may

also influence STM invasion. Lyons et al. (2012) reported increases in STM establishment with higher irrigation frequencies in greenhouse experiments. Borst et al. (2010) found that several light topdressing applications slightly reduced STM severity. Finally, applications of the herbicide carfentrazone can selectively suppress STM in creeping bentgrass (Borst et al. 2010; Kennelly et al. 2010; Thompson et al. 2011b). Different combinations and intensities of these practices are likely to affect the success of STM control measures.

In contrast to the above practices and tools, little is known about the effect of turfgrass cultivation on STM severity in putting greens. Aerification and vertislicing are two common practices utilized by superintendents around the world to manage thatch and compaction, and to increase water infiltration. Both practices create temporary openings in the turfgrass canopy which may be recolonized by either STM or creeping bentgrass; however, it is unclear which species will ultimately occupy the available sites. Personal observations indicate creeping bentgrass may not recolonize an area currently occupied by STM, because stolons and tillers have difficulty penetrating the dense gametophyte. Aerification and vertislicing can create available sites within the gametophyte, which would allow neighboring bentgrass plants to establish; conversely, STM propagules may reside in the available sites and further the infestation.

Each species has characteristics that may increase its competitiveness in filling canopy voids. For example, STM fragments and bulbils are buoyant, and are likely to be transported in water and deposited in the voids. On the other hand, creeping bentgrass stolons typically fill such voids in 1 to 2 weeks under optimal growing conditions. Furthermore, aerification and/or vertislicing may increase the competitiveness of STM and creeping bentgrass in different ways:

- 1) With regard to STM, both practices are likely to dislodge gametophore fragments and bulbils,

possibly increasing STM propagule availability; 2) with regard to creeping bentgrass, both practices may decrease compaction and increase soil-oxygen levels, thereby promoting bentgrass growth. Unfortunately, no published research has evaluated their effects on an existing STM infestation.

When used in conjunction with aerification or verticiling, applications of sand topdressing may give creeping bentgrass a competitive advantage in colonizing available sites by suppressing STM growth (Borst et al. 2010). However, many superintendents do not utilize a frequent topdressing program for a variety of reasons: 1) sand removed during mowing damages mower reels and bedknives; 2) inadequate equipment and labor to spread and store sand; 3) budgetary restrictions; and/or 4) thatch accumulation may be minimal due to a short growing season.

Applications of carfentrazone have been shown to be highly effective at suppressing STM growth (Borst et al. 2010; Kennelly et al. 2010; Thompson et al. 2011b), and therefore may also be a valuable tool for shifting the population dynamics in favor of creeping bentgrass, when aerification and/or verticiling are implemented.

Therefore, the objective of our study was to determine the effect of cultivation, with and without light, frequent topdressing and carfentrazone applications, on an existing STM infestation in a creeping bentgrass putting green. We hypothesized significant cultivation  $\times$  herbicide, and cultivation  $\times$  topdressing treatment interactions, wherein cultivation would reduce STM cover when used in conjunction with carfentrazone or topdressing, but would result in an increase in STM cover when implemented without the herbicide.

## Materials and Methods

### *Site Characteristics and Plot Maintenance.*

A 2-yr field experiment was conducted from October 2012 to October 2014 at the Rocky Ford Turfgrass Research Center in Manhattan, KS. Treatments were applied to a ‘Penncross’ creeping bentgrass (*Agrostis stolonifera* L.) putting green with an existing STM infestation. The green utilized a sand-based rootzone containing 95% sand and 5% soil with a pH of 8.0. The area was walk-mowed 6 days per week from March to November using a Toro Greensmaster Flex 21 (The Toro Company, Bloomington, MN) at a bench cutting height of 3.0 mm. Irrigation was hand-applied to ensure even distribution and typically applied every 1 to 2 days from May-September as needed to prevent creeping bentgrass wilt. Plots were fertilized from April to October and received a total of 171 kg N ha<sup>-1</sup>. An 18-9-18 granular fertilizer (Contec DG, Maumee, OH) was applied at 36 kg N ha<sup>-1</sup> in the spring and fall when cultivation treatments were administered. Additionally, foliar applications of soluble N (46-0-0) and liquid 0-2-2 (LebanonTurf, Lebanon, PA) were applied every two weeks from May to September at a rate of 9.8 kg N ha<sup>-1</sup> and 0.17 kg K ha<sup>-1</sup>, respectively. A tank-mix of Emerald<sup>®</sup> [boscalid, 3-pyridinecarboxamide, 2-chloro-N-(4'-chloro(1,1'-biphenyl)-2-yl), BASF Corp. Durham, NC] and Triton Flo<sup>®</sup> [triticonazole, 5-[(4-chlorophenyl)methylidene]-2,2-dimethyl-1-(1,2,4-triazol-1-ylmethyl)cyclopentan-1-ol, Bayer Environmental Science, Research Triangle Park, NC] at rates of 320 and 286 g a.i. ha<sup>-1</sup>, respectively, was applied on 10 May, 24 June, 31 July, and 6 Sept in 2013 and 29 April, 31 May, 11 July, and 14 Sept in 2014 to control dollar spot and brown patch. Acelepryn<sup>®</sup> [chlorantraniliprole, 3-Bromo-N-[4-chloro-2-methyl-6-[(methylamino)carbonyl]phenyl]-1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide, DuPont,

Wilmington, DE] was applied at 59.0 g a.i. ha<sup>-1</sup> on 13 June and 24 August in 2013 and on 1 July in 2014 to control black cutworms.

### ***Experimental Design and Treatments***

A randomized complete-block design using three blocks and a split-plot treatment structure was used to evaluate three main effects: topdressing (2 levels), herbicide (2 levels), and cultivation (4 levels). Treatments were applied to the same plots in both study years because we were interested in the cumulative effect over time on STM cover. Whole plots measured 3.7 × 3.7 m, and received either infrequent or frequent topdressing applications. The infrequent topdressing treatment consisted of 0.8 L m<sup>-2</sup> of sand applied directly after cultivation treatments, and the frequent treatment consisted of 0.8 L m<sup>-2</sup> of sand applied directly after cultivation treatments + 0.4 L m<sup>-2</sup> of sand applied every two weeks from May to September in 2013 and 2014. Sand was applied using a handheld shaker jar. The 0.9 × 1.2 m subplots contained a 4 (cultivation) × 2 (herbicide) factorial. Cultivation treatments were applied during fall and spring of each study year, specifically on 1 October 2012, 29 March 2013, 8 October 2013, and 7 April 2014. The four cultivation treatments were: 1) 13 mm dia. hollow-tine aerification at low intensity (3.9% surface disruption, SD); 2) 13 mm dia. hollow-tine aerification at high intensity (7.2% SD); 3) vertislicing; and 4) no cultivation. Aerification treatments were applied using a Toro ProCore<sup>®</sup> (648, The Toro Company, Bloomington, MN) operating at 3000 rpm. Ejected cores were removed with a shovel, and dry sand was hand-broomed (Model# 829, The Libman Company, Arcola, IL) into applicable plots until holes were completely filled. Vertislice treatments were applied using a Billy Goat<sup>®</sup> Power Rake (PR550H, Billy Goat Industries, Lee's Summit, MO), that cut 1.5 mm wide slits on 5 cm centers at a depth of 8 mm in the turfgrass surface. The herbicide factor contained two levels: 1) carfentrazone applied at 111 g a.i. ha<sup>-1</sup> one

week before, and one week after cultivation treatments were administered (i.e., four applications per year); and 2) no herbicide. Herbicide applications were made using a two-nozzle CO<sub>2</sub> powered backpack sprayer equipped with TeeJet XR8002VS nozzles, and calibrated to deliver a spray volume of 342 L ha<sup>-1</sup>. All herbicide applications included a nonionic surfactant applied at a 0.25% v/v. Silvery-thread moss is poikilohydric and has the potential to be in a dormant state if extracellular water is not present. Therefore, 2.5 mm of irrigation were applied the night before carfentrazone applications were made to ensure the STM would be actively photosynthesizing.

### ***Data Collection and Analysis***

Percent moss cover was obtained using a 0.9 × 1.2 m rating grid containing 330 intersections on 5 × 5 cm centers. Application of carfentrazone causes rapid necrosis of the gametophyte tissue; however, the gametophyte carcass does not breakdown and regrowth occurs several weeks later (Personal observations). Therefore, a positive grid count was recorded if a STM gametophyte was situated directly under an intersection, regardless of whether it was green and healthy, or black/brown and desiccated. Initial moss cover was determined for each plot on 24 September 2012, one week before any treatments were applied. Subsequent ratings were recorded on March 21, 2013, October 1, 2013, April 1, 2014, and October 1, 2014. Moss cover in individual plots ranged from 20 to 60 % at study initiation, therefore, percent change in moss cover at subsequent rating dates was determined by comparing moss cover in each plot to its initial value. This was the same approach used by Kennelly et al. (2010) and Thompson et al. (2012a). The calculation was: % change in moss cover = [(grid count at rating date / grid count at trial initiation) × 100]-100. Negative values indicate moss cover decreased.

A log transformation [ $x' = \log(x + 1)$ ] was needed to improve homogeneity of residual variances; however, the transformation cannot be executed with negative values. Therefore,

percent cover data were scaled to complete the transformation. Log transformed data were subjected to ANOVA using the PROC MIXED procedure in SAS (SAS software, Version 9.4, 2013, SAS Institute Inc., Cary, NC). Means were separated using Fisher's protected least significant difference ( $P \leq 0.05$ ). Nontransformed data are presented, but statistical inferences are based on transformed data.

## **Results and Discussion**

Several treatments were effective at reducing STM cover over the course of the 2-yr field study; however, no treatment combination completely eradicated STM.

### ***Cultivation***

Aerification and vertislicing are practices often used by golf course superintendents which create open spaces, or voids, in the turfgrass canopy; however, it was unknown if these voids would be colonized by STM or creeping bentgrass. Our hypothesis that cultivation would only decrease STM cover when combined with herbicide use or topdressing turned out to be incorrect; both vertislicing and 3.9% SD aerification reduced STM cover compared to the untreated control by the end of the study (Figure 2.1). While the reduction in STM cover was greatest where carfentrazone was used in tandem with cultivation (data not shown), cultivation treatments reduced STM cover irrespective of herbicide use or topdressing (Table 2.1). This is the first research describing the effects of cultivation on a STM infestation. The fact that cultivation did not increase STM cover over the course of this 2-yr study will be welcome news to golf course superintendents, considering the widespread adoption of these practices throughout the world. The fact that cultivation reduced STM cover in the absence of

carfentrazone is, perhaps, even more important to golf course superintendents, because superintendents in some parts of the world do not have access to carfentrazone.

Notably, the effect of cultivation was not significant until after the treatments had been administered three times (Table 2.1). Cultivation is known to have positive effects on rootzone properties (Dernoeden 2012; McCarty et al. 2005), and it seems likely that the cumulative beneficial effects of cultivation treatments helped the bentgrass to have a competitive advantage in filling the canopy voids. While the 3.9% SD aerification and vertislicing treatments reduced STM cover by the spring and fall of 2014 compared to the untreated, the 7.2% SD aerification treatment resulted in slightly less STM cover, and was not different from the untreated (Figure 2.1). Currently, it is unclear why the 7.2% SD treatment was not as effective as other cultivation treatments and warrants further research to determine if an optimal SD for STM control exists.

### *Carfentrazone*

The effect of carfentrazone was highly significant at every rating date throughout the study (Table 2.1). On average, a 20% reduction in STM cover was observed after two applications of carfentrazone at a rate of 111 g a.i. ha<sup>-1</sup> (Figure 2.2). These results are similar to those reported by Kennelly et al. (2010) and Borst et al. (2010), who reported a 39 and 36% reduction in STM severity, respectively, when treated with carfentrazone. No negative effects to bentgrass color or quality were observed following the split applications of carfentrazone; however, this herbicide, a protoporphyrinogen oxidase inhibitor (Senseman 2007), cause rapid necrosis in the gametophore tips by 2 to 3 DAT. A total of eight applications of carfentrazone were applied throughout the duration of the study, but on average, only an 80% reduction in moss severity was observed (Figure 2.2). Bryophytes, such as silvery-thread moss, have been reported to have a slower decomposition rate compared to vascular plants (Scheffer et al. 2001).

Carfentrazone is very effective at injuring STM; however, the necrotic gametophyte is able to resist decay, and regrowth often occurs directly from the injured shoots in the weeks following the initial application.

Herbicide timing often has a dramatic effect on the efficacy of herbicide applications (Johnson et al. 2002; Raudenbush and Keeley 2014; Reicher and Weisenberger 2007). In this study, the split-applications of carfentrazone were strategically made one week before and one week after cultivation treatments were administered. Our intention was that the first of the split-applications would injure the STM gametophyte, allowing creeping bentgrass to fill the voids created by the cultivation treatments, while the second application (two weeks later) would control any dispersed plant material. This research supports previous findings showing that carfentrazone is a valuable tool that can temporarily shift stand dynamics in favor of the desirable turfgrass species; however, it not likely to completely eradicate STM. As noted previously, the greatest reductions in STM cover were observed when carfentrazone was used in conjunction with cultivation treatments (data not shown).

### ***Topdressing***

Theoretically, topdressing in conjunction with cultivation would shift stand dynamics in favor of creeping bentgrass, by partially covering STM (Borst et al. 2010) and thereby reducing its photosynthesis rate. However, in our study, light frequent topdressing did not affect STM cover at any rating date throughout the experiment (Table 2.1). These results differ from Borst et al. (2010), who reported a 39% reduction in STM cover from four applications of sand topdressing. The whole plots receiving frequent topdressing did have enhanced turfgrass color and quality (data not shown). The lack of effect on STM cover in our study may have been a consequence of the statistical design we employed. Because the topdressing factor was assigned

to the whole plots in our split-plot treatment structure, this factor had only two denominator degrees of freedom in the ANOVA. We chose this treatment structure for two main reasons: 1) Concern that assigning the topdressing factor to individual small plots in a 2 (topdressing)  $\times$  4 (cultivation)  $\times$  2 (herbicide) factorial design would create a checkerboard effect, in which the canopy of plots receiving frequent topdressing would become elevated as the study progressed, causing the mower to scalp plot edges and introduce unwanted variation; and 2) We were highly interested in cultivation and herbicide effects, and their interaction, and our treatment structure resulted in more statistical power for those factors/interactions.

### ***Recommendations***

Superintendents confronted with STM infestations should use a multifaceted approach with two main goals: 1) reduce the size of the current infestation, while 2) preventing the future establishment of dispersed propagules. Our research demonstrates that hollow-tine aerification or vertislicing, in tandem with split applications of carfentrazone, effectively accomplishes these goals. Unfortunately, cultivation disrupts playability and would have to be performed with moderate intensity to reduce STM cover. Silvery-thread moss is typically a problem in select areas of the putting green, and if twice yearly cultivation is not feasible, we recommend selectively implementing cultivation in those areas where STM is most prevalent. Greatest reductions in STM cover will likely be attained by combining cultivation and carfentrazone. Although light, frequent topdressing did not reduce STM cover in our study, other research supports its inclusion as part of a comprehensive STM management strategy. A light, frequent topdressing program should help reduce survival of dispersed fragments and bulbils by directly burying the propagules. Lastly, this research may be most applicable to putting greens with large (e.g., >10%) STM infestations. It is unclear if very small infestations (e.g., 1% or less) would

respond to cultivation in the same manner. Investigating such a scenario could be the subject of future research.

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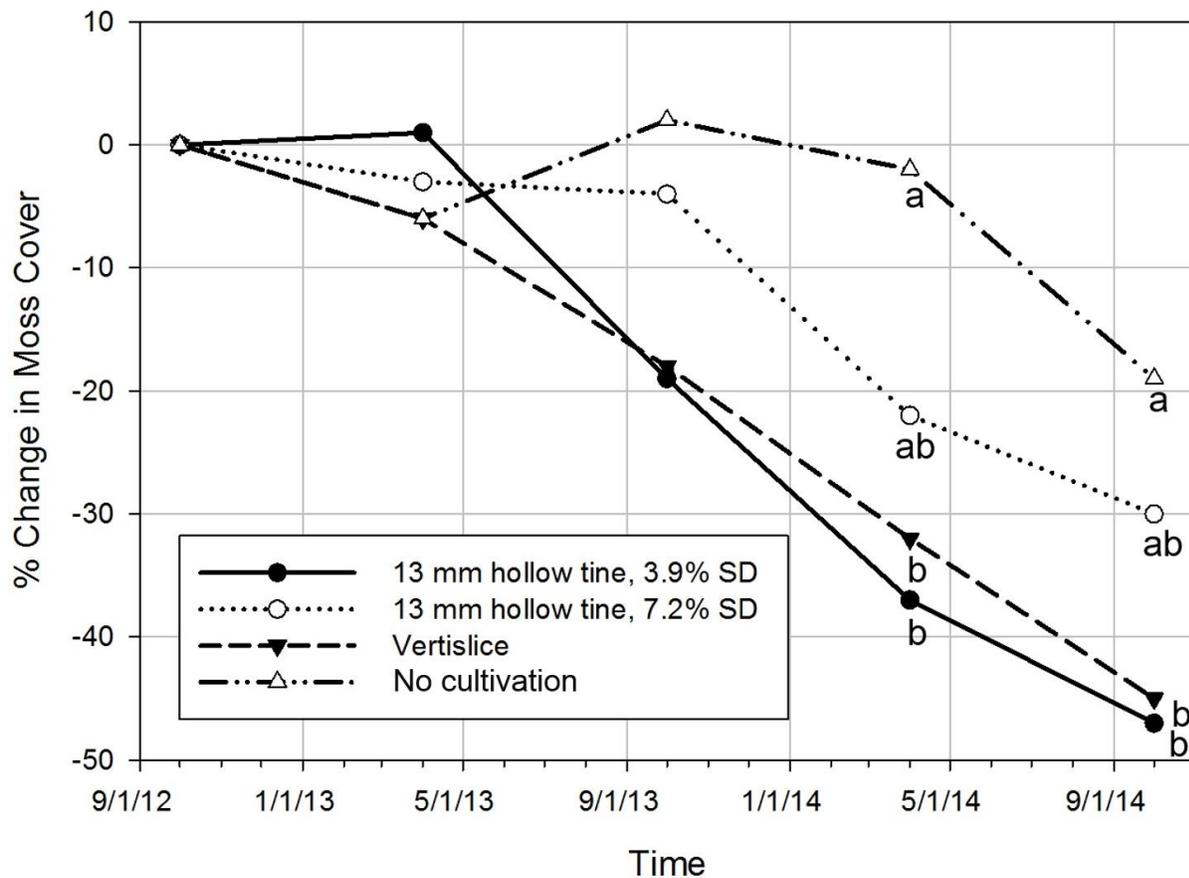


Figure 2.1 Effect of cultivation treatment on silvery-thread moss cover in a creeping bentgrass putting green in 2013 and 2014. The 3.9% SD and 7.2% SD indicate hollow tine aeration at low and high surface disruption, respectively. Vertislice treatments involved cutting 1.5 mm wide slits on 5 cm centers to a depth of 8 mm in the turfgrass surface. Cultivation treatments were performed each spring and fall starting on October 1, 2012 and ending on April 7, 2014. Means followed by the same letter on individual rating dates are not significantly different ( $P < 0.05$ ) according to Fisher's Protected LSD.

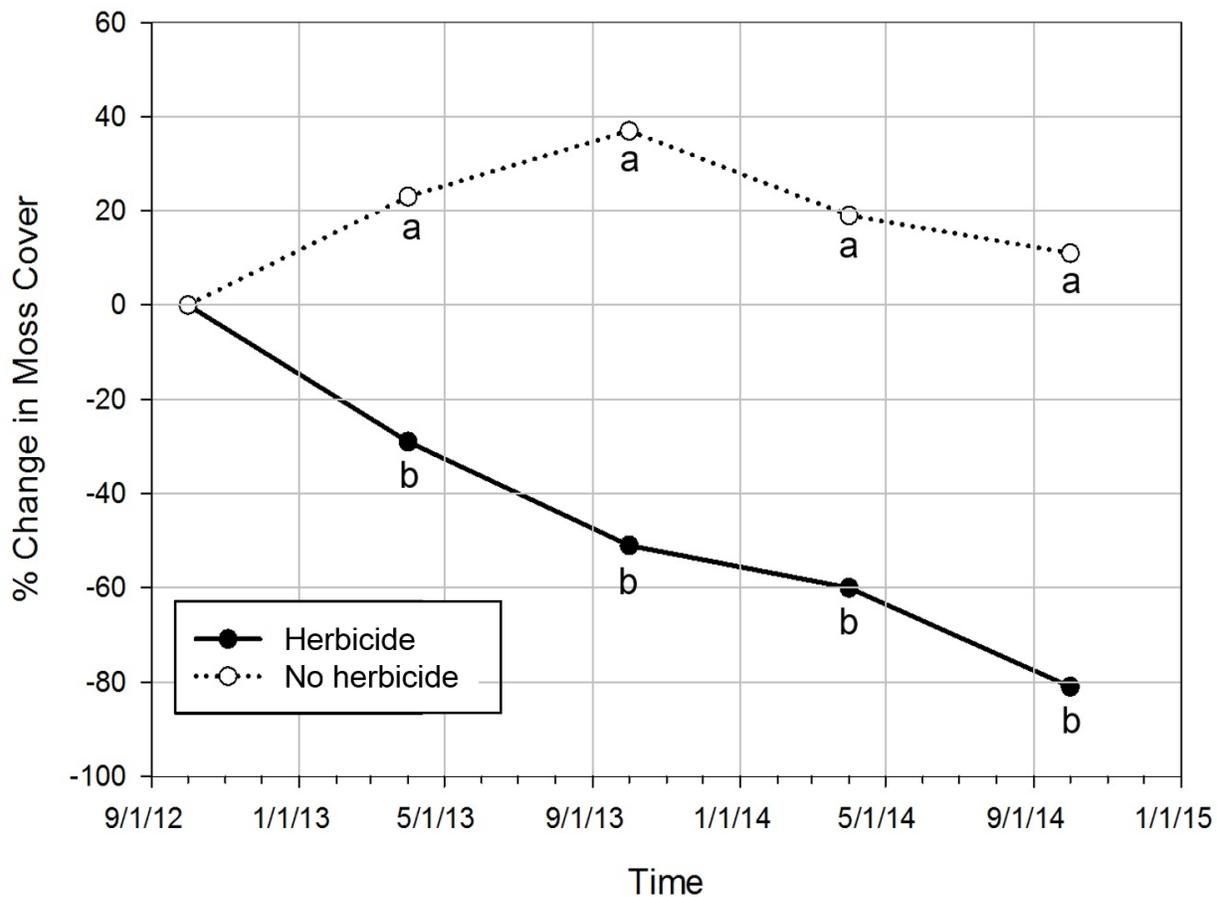


Figure 2.2 Effect of carfentrazone on silvery-thread moss cover in a creeping bentgrass putting green in 2013 and 2014. Herbicide applications began on September 25, 2012 and the last application was on April 14, 2014. Each spring and fall a split application was applied at 111 g a.i ha<sup>-1</sup>, two weeks apart (four total applications per year). Means followed by the same letter on individual rating dates are not significantly different ( $P < 0.05$ ) according to Fisher's Protected LSD.

Table 2.1 ANOVA for percent change in silvery-thread moss cover when combinations of topdressing, herbicide, and cultivation were applied to a creeping bentgrass putting green in Manhattan, KS.

Source	Num df	Den df	<i>P</i> -value Associated with % Change in Moss Cover			
			2013		2014	
			Spring	Fall	Spring	Fall
Topdressing	1	2	0.5365	0.7346	0.8991	0.6599
Herbicide	1	28	<.0001	<.0001	<.0001	<.0001
T × H	1	28	0.4842	0.2038	0.1423	0.4666
Cultivation	3	28	0.6735	0.1006	0.0131	0.0070
T × C	3	28	0.2118	0.6961	0.4654	0.5638
H × C	3	28	0.6653	0.4972	0.7981	0.3725
T × H × C	3	28	0.0679	0.9044	0.8055	0.2375

# **Chapter 3 - Effect of Nitrogen Source and Spray Volume on the Establishment and Colonization of Silvery-Thread Moss (*Bryum argenteum* Hedw.) in Putting Greens**

## **Abstract**

During the growing season, golf course superintendents often fertilize putting greens with low nitrogen rates on a weekly or biweekly interval, using foliar applications of soluble N sources such as ammonium sulfate (AMS) or urea. Silvery-thread moss (STM) is ectohydric; consequently, we hypothesized that STM would be injured from the higher partial salt index of AMS compared to urea, and that injury would be exacerbated by low spray volumes. Therefore, the objectives of this study were to: 1) determine if STM growth is reduced by AMS compared to urea, and 2) understand the effect of spray volume on STM growth, when using a high-salt-index N source. In greenhouse studies, AMS or urea were applied weekly at 4.4 lbs N acre<sup>-1</sup> to nascent STM growing in pots. The N sources were applied at three spray volumes: 11, 44, and 110 gal H<sub>2</sub>O acre<sup>-1</sup>. Percent moss cover was determined weekly from digital images, and after seven weeks, gametophytes were harvested, dried, and weights recorded. Contrary to our hypothesis, AMS increased STM cover and dry weight compared to urea and an untreated control. Furthermore, spray volume did not affect STM cover at any rating date. Follow-up field studies were conducted on putting greens at Rocky Ford Turfgrass Research Center and Colbert Hills Golf Course. Although results were less consistent in the field, AMS again generally increased STM cover compared to urea and the untreated control. Superintendents managing STM infestations should limit or avoid use of AMS as an N source.

## Introduction

The establishment and spread of silvery-thread moss (STM; *Bryum argenteum* Hedw.) in golf course putting greens is likely affected by fertilization practices along with other factors (e.g., irrigation, mowing height, infiltration, drainage, etc.) (Raudenbush et al., 2015), but the role of nitrogen (N) fertilization is unclear. Nitrogen is important for healthy putting green turf, and is often applied to greens at 4.4 to 8.8 lbs N acre<sup>-1</sup> every 10 to 14 days during the growing season. Some have speculated that low N fertility encourages the invasion of STM in putting greens (Cook et al., 2002; Hummel, 1994); this could occur because a low N environment often causes poor turfgrass density, providing voids for opportunistic weeds (Johnson and Bowyer, 1982; Murray et al., 1983). However, with some weeds, high N levels increase weed competitiveness (e.g., annual bluegrass) (Lodge and Lawson, 1993). To date, the effect of N level on STM in putting greens has not been adequately researched.

Silvery-thread moss is ectohydric, so it cannot extract water and nutrients from within the soil profile; rather, it absorbs water and soluble nutrients foliarly (Glime, 2007). Consequently, the source of N may dramatically impact STM growth. In particular, the competitiveness of STM may be enhanced if soluble nutrients are applied, compared to insoluble nutrients. Recent research supports this idea: Kennelly et al. (2010) supplied 13 lbs N acre<sup>-1</sup> every two weeks using two N sources: liquid urea (46-0-0) and a granular organic poultry litter (8-1-3). They reported a 200% increase in STM cover in plots receiving liquid urea compared to the granular organic N source. Similarly, Thompson et al. (2011) found plots fertilized with liquid urea had a 47% and 50% increase in STM cover compared to plots receiving IBDU and organic poultry litter, respectively.

Unfortunately, applying insoluble nutrients, specifically N, may not be preferred by superintendents for a variety of reasons: 1) Some insoluble granular products emit a foul odor

after application; 2) granular fertilizers may stick to the rollers on reel mowers and create a marcelling effect; 3) and granular fertilizers may be directly removed during mowing, reducing their effectiveness. By contrast, soluble N can be added to a spray tank and applied in conjunction with pesticide and plant growth regulator applications. Therefore, research regarding soluble N sources and their effect on silvery-thread moss infestations would be extremely valuable to superintendents.

Urea and ammonium sulfate (AMS) are two soluble N sources commonly used by superintendents. Ultimately, both N sources are converted from  $\text{NH}_4^+$  to  $\text{NO}_3^-$  in the soil via nitrification (Turgeon, 2004); however, agronomists often recommend AMS as the primary N source when combating a high rootzone pH. This is because the nitrification of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  in soil produces two net moles of  $\text{H}^+$  for every mole of nitrified  $\text{NH}_4^+$ . Conversely, urea only produces one net mole of  $\text{H}^+$  for every mole of ammoniacal N because urea hydrolysis produces a competing mole of  $\text{OH}^-$  (Lungu and Dynoodt, 2008). Ammonium sulfate may be the ideal nitrogen source when attempting to reduce rootzone pH, but it has a higher potential than urea to burn turfgrass leaves, due to its higher partial salt index. A high partial salt index can create an osmotic gradient, which potentially could draw water directly from the cells of turfgrass leaves, causing discoloration (Stiegler et al., 2013).

With respect to STM, this effect on osmotic potential is of interest because bryophytes are especially susceptible to osmotic shock (Glime, 2007). As usually practiced by superintendents, the spraying of high-salt-index fertilizers at low rates may result in little or no foliar burn, because superintendents typically apply fertilizers, fungicides, and plant growth regulators at relatively high spray volumes ( $> 60 \text{ gal acre}^{-1}$ ). Conversely, a low spray volume would result in a higher concentration of salts in individual spray droplets, which could have a

detrimental effect on STM. In a different bryophyte, Voth (1943) found the growth of *Marchantia polymorpha* decreased as the salt concentration of nutrient solutions increased.

Because of its higher partial salt index, it is plausible that substituting AMS for urea would be detrimental to STM, and injury would likely be enhanced at lower spray volumes. Therefore, the two objectives of this research were to: 1) determine if STM growth could be reduced by substituting AMS for urea as a N source and; 2) understand if a lower spray volume would reduce the growth of STM when used with a high-salt-index N source. We hypothesized that AMS would injure STM regardless of spray volume, but that the injury would be enhanced at lower spray volumes.

## **Materials and Methods**

### ***Greenhouse Study Material Preparation***

A greenhouse study was conducted in the Throckmorton Plant Science Center at Kansas State University, Manhattan, KS to determine the effects of N source and spray volume on STM growth. Greenhouse temperature ranged from 64° to 78° F (Hobo ProV2, Onset Computer Corporation, Bourne, MA) throughout the duration of the study. The STM population used in the experiment was a colony originally collected from a research green at Rocky Ford Turfgrass Research Center in Manhattan, KS. The population was clonally propagated in the greenhouse for several months prior to trial initiation to obtain sufficient plant material. The propagated plant material was removed from the greenhouse and allowed to air-dry in the laboratory at 68° F for 7 days. After 7 days, 0.2 oz of dried plant material were placed in a coffee grinder (Krups F20342, Millville, NJ) and ground for ~ 6 s until the plant material was sufficiently shredded.

PVC containers (4 inches dia. by 8.5 inches deep) were filled with pea gravel to a height of 2 inches, and then filled with 6 inches of moist sand conforming to United States Golf Association specifications for a putting green rootzone. Containers were filled to field capacity and allowed to drain several times to encourage settling, and additional sand was added as needed to position the sand 0.75 inch from the top of the pot. After pots drained for 24 h, each pot was planted with 0.03 oz of ground STM material. Plant material was evenly spread over each individual pot and then immediately watered with a misting nozzle (Dramm 610SF, Manitowoc, WI) until saturated. The misting nozzle was continuously moved while watering the pots to prevent puddling of water at the soil surface. Puddling would have allowed the ground gametophyte material to float and consequently migrate towards the outer edge of the pots, reducing uniformity. Pots were watered three times per day for 10 days, and then once per day for the remainder of the study. Silvery-thread moss gametophores began actively growing after 7 days. A total of 36 pots were needed for each of the two runs of the experiment; however, 50 pots were planted for each run. After 10 days, percent moss cover was determined for all 50 pots by the methods described below, and the 36 pots with the most homogeneous cover were selected for each run.

### ***Greenhouse Experimental Design and Data Collection***

Treatments were applied approximately every 7 days in the greenhouse for a total of seven applications. Nitrogen source treatments included AMS, urea, and untreated. Ammonium sulfate and urea were applied at a rate of 4.4 lbs N acre<sup>-1</sup> per week. Additionally, dibasic potassium phosphate was used to supply all three treatments with 0.37 lbs P and 1.8 lbs K acre<sup>-1</sup> per week. Spray volumes were 11, 44, and 110 gal H<sub>2</sub>O acre<sup>-1</sup>, which were obtained using TeeJet XR8001EVS, XR8004EVS, and XR8008EVS nozzles (Spraying Systems Co., Wheaton, IL),

respectively. Applications were made using a single nozzle boom and a metronome was used to maintain steady walking speed. The experiment used a completely randomized design with four replicates and a two-way factorial treatment structure to evaluate the effect of nitrogen source (factor a) and spray volume (factor b).

Percent moss cover was determined weekly using overhead images, and those images were batch-processed using the methods described by Richardson et al. (2001). Images were obtained using a Nikon D3000 (Nikon Co., Tokyo, Japan) digital single-lens reflex camera affixed to a custom-built lightbox (20 × 28 × 24 inches) containing four 6500 K-temperature fluorescent light bulbs (model ESL23TM/D, Feit Electric Co., Pico Rivera, CA). The camera utilized a shutter speed of 1/125 s, aperture of F5.6, ISO 800, and focal length of 50 mm. Images were saved as JPEG format because light conditions were uniform and well-controlled throughout the experiment. Percent cover was extracted from images using SigmaScan Pro (Version 5.0, SPSS, Chicago, IL). The program was able to selectively identify individual STM gametophores utilizing a hue range of 45-75 and a saturation range of 50-100.

After trial completion, moss gametophytes were harvested, dried at 170° F for 48 h, and dry weights were recorded. Percent moss cover and gametophyte dry weights were subjected to analysis of variance using the GLM procedure of Statistical Analysis System (SAS) software (Version 9.4; SAS Institute Inc., Cary, NC). Means were separated using Fisher's protected least significant difference (LSD) test ( $P \leq 0.05$ ).

### ***Field Study Plot Maintenance***

In 2014, field studies were conducted from May to October at Rocky Ford Turfgrass Research Center and Colbert Hills Golf Club in Manhattan, KS. The Rocky Ford putting green utilized a sand-based rootzone and contained 'Declaration' creeping bentgrass (*Agrostis*

*stolonifera* L.), which was mowed six days per week with a flex cutting unit set to a bench cutting height of 0.115 inch. The Colbert Hills green was of California-style construction and contained ‘Penn G-2’ creeping bentgrass. It also was mowed six days per week, but with a triplex mower set to a bench cutting height of 0.115 inch. Both sites were irrigated every 2 to 3 days at 100% ET replacement estimated from onsite weather stations and the FAO-56 Penman-Monteith equation.

### ***Field Study Experimental Design and Data Collection***

Spray volume had minimal effect on STM growth in the greenhouse (Table 3.1); therefore, to make efficient use of limited experimental units, field studies only evaluated the effect of N source on STM infestation. At both locations, a randomized complete-block design with five blocks and a one-way treatment structure was used to evaluate N sources AMS, urea, and untreated. Blocks were constructed by placing three plots with similar initial percent cover in the same block to improve homogeneity of experimental units. Similar to greenhouse experiments, treatments were applied every 7 days, but for a longer time period; specifically, from May 15 to October 15. Ammonium sulfate and urea were applied at 4.4 lbs N acre<sup>-1</sup>. Dibasic potassium phosphate supplied all treatments with 0.37 lbs P and 1.8 lbs K acre<sup>-1</sup>. After fertilizers were dissolved into solution, applications were made with a single nozzle (TeeJet XR8004EVS) CO<sub>2</sub> powered backpack sprayer operating at 30 psi to deliver a spray volume of 44 gal acre<sup>-1</sup>. Initial STM cover at Rocky Ford ranged from 5-15% cover with a mean cover of 9%, and the STM cover at Colbert Hills ranged from 5-21% with a mean cover of 13%. Silvery-thread moss cover of the 3 × 3 ft plots was determined using a rating grid containing 961 intersections on 1 inch centers. A count was registered if a STM gametophyte was positioned directly under an intersection. Percent change in moss cover at subsequent rating dates was

determined by comparing moss cover in each plot to its initial value. This was the same approach used by Kennelly et al. (2010) and Thompson et al. (2011). The calculation was: % change in moss cover = [(grid count at rating date / grid count at trial initiation) × 100]-100. Negative values indicate moss cover decreased.

Normality of residue variances were examined using the UNIVARIATE and GPLOT procedures in SAS. Percent change in moss cover at Rocky Ford was normal; however, a log transformation [ $x' = \log(x + 1)$ ] was needed to normalize cover data from Colbert Hills. Percent moss cover data (transformed and untransformed) were subjected to analysis of variance using the MIXED procedure of SAS and means were separated using Fisher's protected LSD test ( $P \leq 0.05$ ); transformed means were back-transformed for presentation.

## **Results and Discussion**

### ***Effect of N Source in Greenhouse Study***

A significant Run × N-source interaction was present, therefore results from Run 1 and 2 are presented separately. The interaction was likely due to the lower initial moss cover in Run 2 (Figure 3.1), which allowed the untreated plots to increase substantially in STM cover during the first few weeks of Run 2, but not Run 1. Final STM cover under all treatments was slightly higher in Run 2, but overall treatment effects were similar by the end of each run.

Our hypothesis that AMS would be detrimental to STM growth, and that this effect would be exacerbated by low spray volumes, was incorrect; on the contrary, AMS typically increased STM growth in both greenhouse and field trials (Figures 3.1 - 3.5), and spray volume did not affect moss cover (Table 3.1). A low spray volume would only have a negative effect on STM growth if the salt content of the spray solution was sufficiently high to be toxic to STM.

Perhaps the low N rates used in our research, which were chosen because they are representative of the “spoon-feeding” approach used by many golf course superintendents, did not result in a sufficiently high salt content of the spray solution to cause injury to STM. Higher rates of AMS in combination with low spray volumes could possibly cause foliar burn to STM (as sometimes happens with turfgrass), but substantially higher rates of soluble N are not typically used on greens. Additionally, to obtain the lowest spray volume evaluated in this study (11 gal acre<sup>-1</sup>), superintendents would have to drastically increase sprayer ground-speed or use low-volume nozzles. Both options may be problematic, because a slower ground speed is usually necessary when spraying greens to provide the operator ample time to maneuver around intricate green complexes; and secondly, low-volume nozzles have a small orifice that can easily be clogged by water-dispersable-granule or wettable-powder fungicide formulations.

Unlike spray volume, N source was highly significant ( $P < .01$ ) on every rating date in both runs of the experiment (Table 3.1). While both AMS and urea generally increased STM cover compared to the untreated, AMS caused the greatest increases. In Run 1, AMS increased STM cover on all 7 rating dates, and caused greater moss cover than urea on all dates after week 1 (Figure 3.1). Urea increased STM cover compared to the untreated on 4 of 7 rating dates. In Run 2, AMS again increased STM cover compared to the untreated on all 7 rating dates, while urea increased STM cover on 6 of 7 dates. Ammonium sulfate caused greater STM cover than urea on 4 of 7 rating dates, including the last three weeks of the study (Figures 3.1 and 3.2).

In addition to STM cover, dry weight of harvested STM gametophytes was highly affected by N source (Table 3.1). Gametophytes treated with AMS had a 3- and 2-fold increase in dry weight compared to urea and the untreated in Runs 1 and 2, respectively (Figure 3.3). Urea did not significantly increase STM dry weight compared to the untreated in either run of the

experiment. The increased dry weight caused by AMS was the result of longer gametophyte filaments (gametophores), which ultimately reflects increased leaf production. Longer gametophores enable STM to better compete for sunlight in the turfgrass canopy, while crowding out desirable turfgrass species. Additionally, longer gametophores are more likely to be sheered off or dislodged during mowing, which may aid in the dispersal of propagules (Raudenbush et al., 2015).

As noted, our hypothesis that AMS would decrease STM growth was proven false, at least at the low rates used in “spoon-feeding” fertilization programs, and the spray volumes used in this research. However, the fact that AMS actually caused greater STM growth than urea was more surprising. At least four scenarios seem plausible to explain the increase in STM growth with AMS compared to urea in the greenhouse study: 1) Less N uptake occurred with urea because the activity/abundance of urease, on or in STM gametophores, was reduced for unknown reasons (foliar-applied urea is typically hydrolyzed to  $\text{NH}_4^+$  by urease on the leaf surfaces (Stiegler et al., 2013), however, direct uptake of the intact molecule is known to occur in other plants (Wittwer et al., 1963)); 2) Hydrolyzed urea may have volatilized, reducing N uptake; 3) Ammonium sulfate may have reduced the pH of the water film retained by the gametophyte, which positively impacted STM growth (current research is underway by the authors that indicates STM growth is greater when irrigated with slightly acidic water compared to water of neutral or alkaline pH); and lastly, 4) The sulfate supplied by AMS may have positively impacted STM growth. These possibilities should be the subject of future research.

### ***Effect of N Source in Field Study***

Because of the dramatic effect of AMS on STM growth in the greenhouse, field studies were conducted to see if the results could be duplicated in the field. Location was included in the

model and a significant location  $\times$  N-source interaction was present; therefore, results for each location are presented separately. This interaction occurred because the STM infestation at Rocky Ford decreased throughout the summer months, while an increase was observed at Colbert Hills.

The Colbert Hills study (described below) corroborated our greenhouse results; however, in the Rocky Ford study N source did not affect moss cover, although the *P*-value associated with *F* trended downward as the study progressed (Table 3.2). The lack of significance appeared to be due to variation in the distribution of STM over the plot area, and especially an anomalous AMS-treated plot in one block. For example, at the October rating date, AMS treated plots had increased STM cover in 4 of 5 blocks, ranging from 40 to 177%, but the fifth block had a decrease in STM cover. We are confident in the accuracy of our data, considering the large number of intersections in our rating grid (961). Other factors besides N and N source obviously affect STM's competitiveness (Kennelly et al., 2010; Lyons et al., 2012), and some unknown factor caused the decline of STM in the anomalous plot.

At Colbert Hills, N source did affect STM cover at the last two rating dates (Table 3.2 and Figure 3.4). Ammonium sulfate increased STM cover on the last 2 (out of 5) rating dates compared to the untreated, and on the next to last rating date compared to urea (Figure 3.4). By the study's end, AMS increased STM cover by nearly 200%, which was similar to the results reported by Kennelly et al. (2010) with urea. Urea increased STM cover by 55%, but was not different from the untreated at any rating date. By contrast, Thompson et al. (2011) found that foliar-applied urea increased STM severity by 55% compared to the untreated.

Overall, STM cover at Rocky Ford and Colbert Hills was similar at study initiation; however, differences between the two locations became evident as the study progressed. At

Colbert Hills, the size of the infestation did not decrease throughout the study; in contrast, a reduction in STM cover was observed at Rocky Ford during the summer months (Figure 3.5). It is surprising that no decrease in STM cover occurred during summer at Colbert Hills, considering STM utilizes a C-3 pathway for carbon fixation (Crum and Anderson, 1973). Smith (1999) reported maximal photosynthesis rates of *Bryum argenteum* collected from Antarctica at temperatures of 59 to 68° F, and Longton (1981) reported that day/night temperatures of 95/86° F were close to the upper limit for STM survival. The average maximum/minimum air temperature in Manhattan, KS from June 1 through August 30, 2014 was 88/65° F, which makes the increase in STM cover at Colbert Hills even more interesting. However, several researchers have found significant variation among STM populations (Longton, 1981; Horsley et al., 2011), which may explain the differences in STM growth between the two locations.

Lastly, at both locations, an increase in STM cover was observed in September and October (Figure 3.6). Superintendents should consider applying a selective herbicide, such as carfentrazone-ethyl, in the fall to reduce the competitiveness of STM as temperatures decrease. This is especially important considering STM is capable of fixing a significant amount of carbon at temperatures as low as 41° F (Smith, 1999), when the relative growth rate of turfgrass is low.

## **Conclusions**

This research supports previous findings that spraying soluble nitrogen throughout the growing season is likely to increase the competitiveness of STM (Kennelly et al., 2010; Thompson et al., 2011). Additionally, based on our research, superintendents struggling with STM should limit or avoid use of AMS as an N source. Although granular fertilizers have drawbacks for putting green fertilization, previous research demonstrated that granular N-sources

did not increase STM cover in putting greens (Borst et al., 2010; Kennelly et al., 2010; Thompson et al., 2011); therefore, superintendents should consider including them in their N fertilization program, especially in the spring and fall when STM is highly competitive. If superintendents choose to spray soluble N, they should be aware that STM will be more competitive and should implement a STM control program. Such a program may include selective herbicide use (Kennelly et al., 2010), along with cultural practices such as reduced irrigation frequency (Lyons et al., 2012), cultivation (Raudenbush and Keeley, 2014), and topdressing (Borst et al., 2010).

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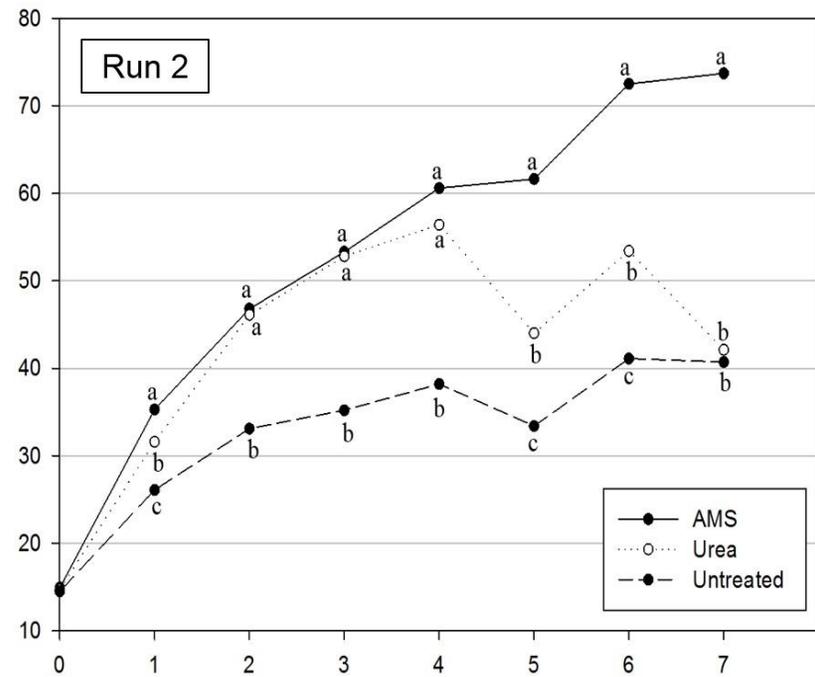
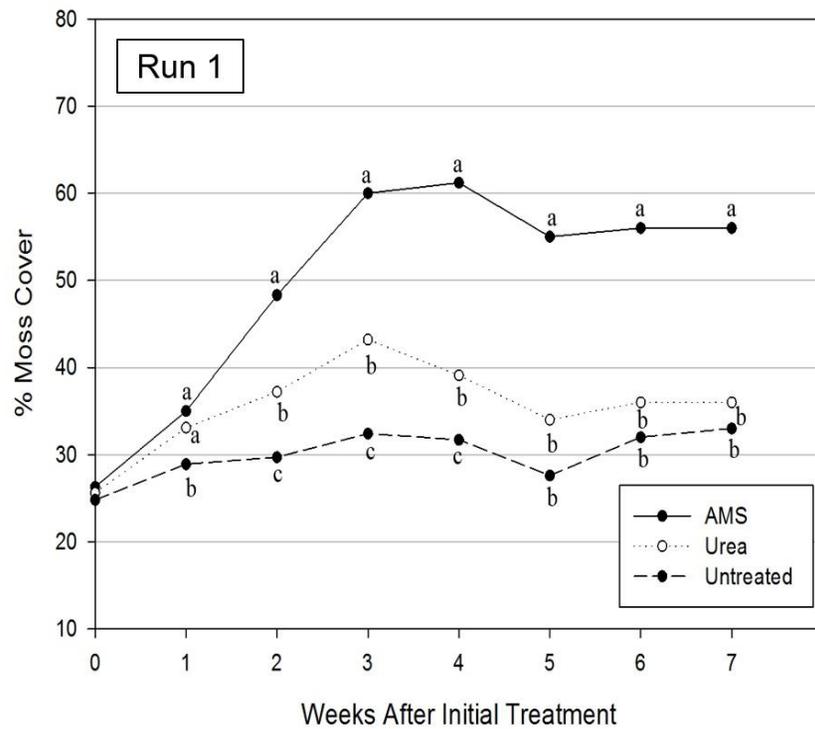


Figure 3.1 Effect of ammonium sulfate (AMS) and urea sprayed weekly at 4.4 lbs N acre<sup>-1</sup> for seven weeks on silvery-thread moss (*Bryum argenteum* Hedw.) cover in the greenhouse in Run 1 and 2. Means followed by the same letter on individual rating dates are not significantly different ( $P < 0.05$ ) according to Fisher's Protected LSD test.

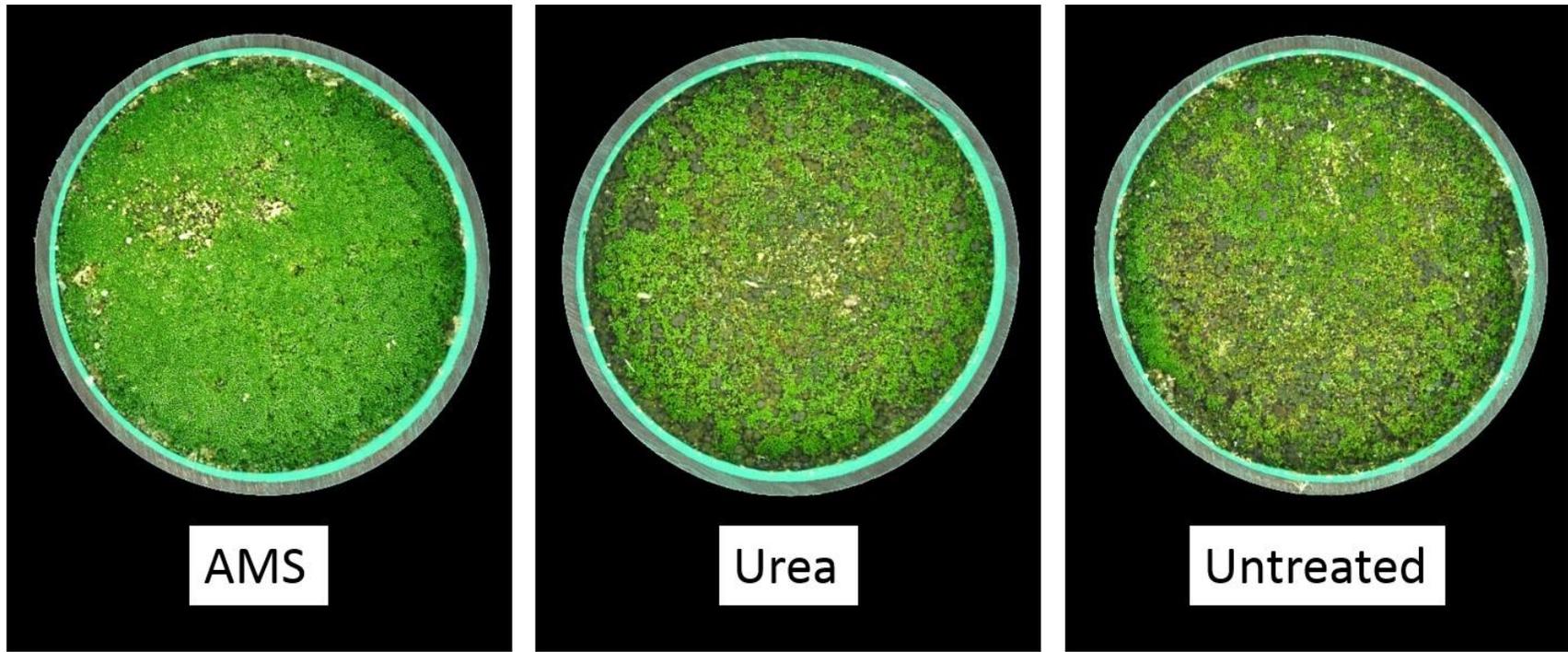


Figure 3.2 Lightbox images showing effect of ammonium sulfate (AMS) and urea when applied at  $4.4 \text{ lbs N acre}^{-1} \text{ wk}^{-1}$  on silvery-thread moss (*Bryum argenteum* Hedw.) for seven weeks, at a spray volume of  $44 \text{ gal acre}^{-1}$ , in Run 2 of greenhouse study. Images captured at seven weeks after initial treatment.

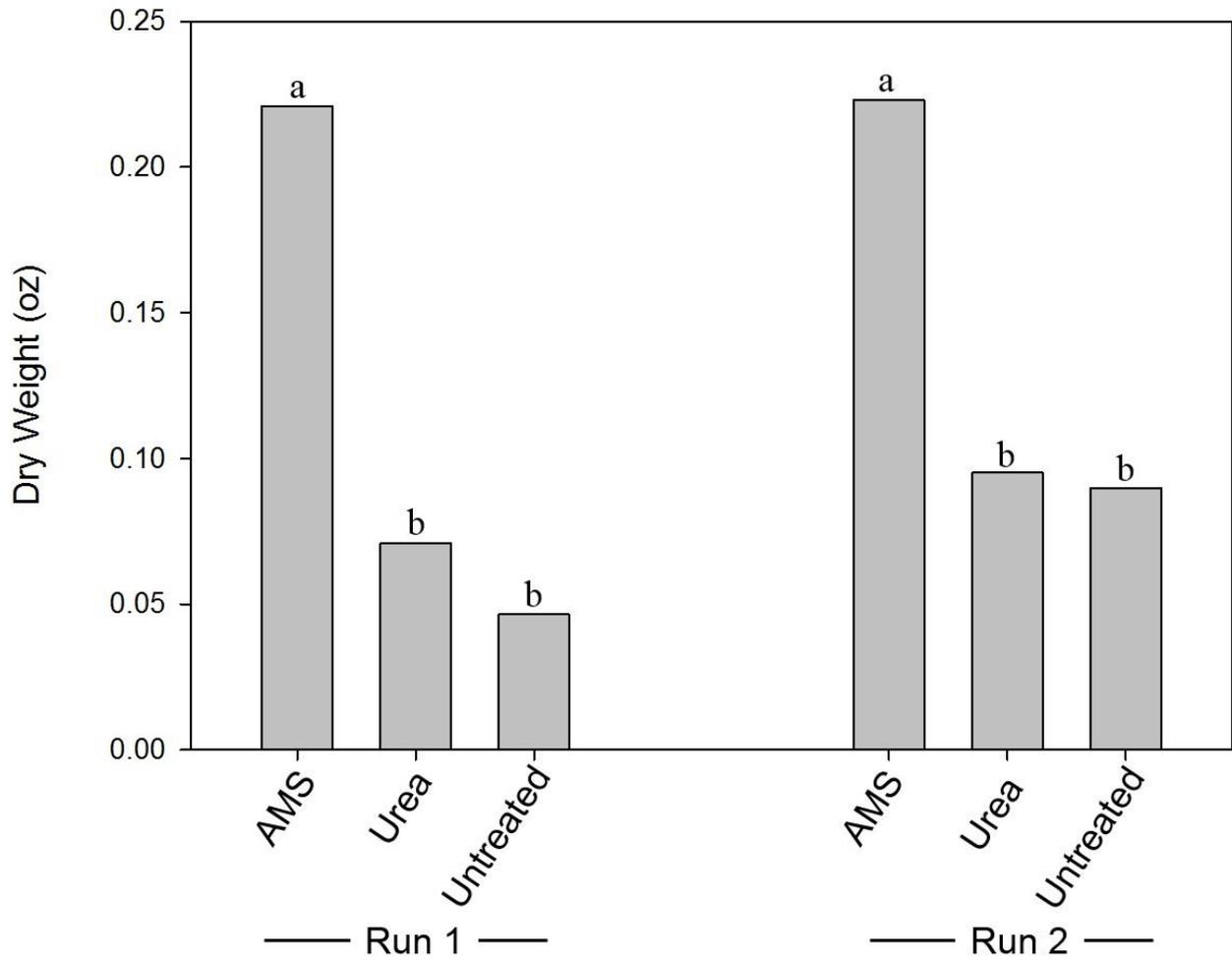


Figure 3.3 Effect of ammonium sulfate (AMS) and urea sprayed weekly at 4.4 lbs N acre<sup>-1</sup> for seven weeks on silvery-thread moss (*Bryum argenteum* Hedw.) dry weight at experiment termination in the greenhouse. Within each run, treatments with the same letter above the bar are not significantly different ( $P < 0.05$ ) according to Fisher's protected LSD test.

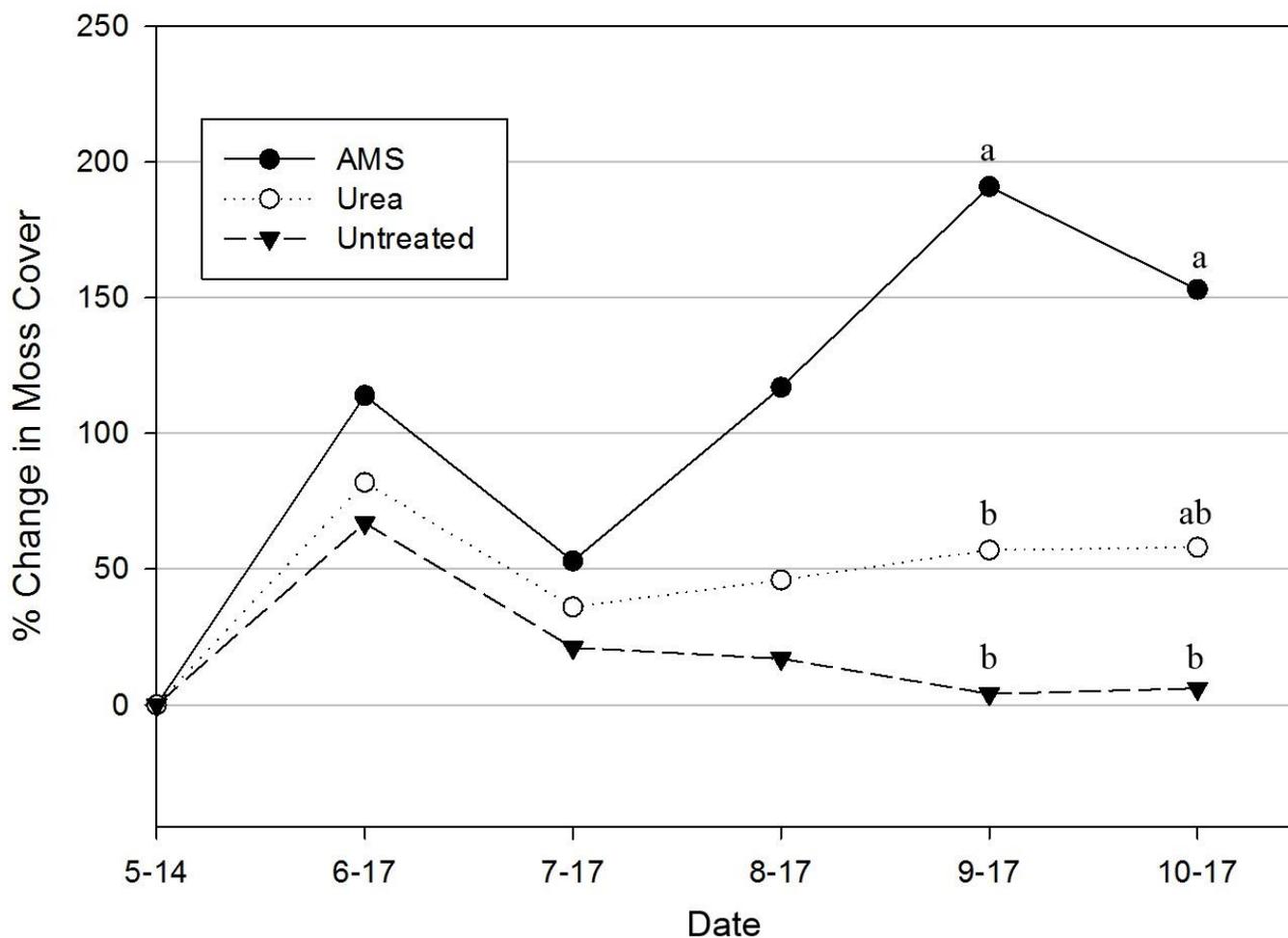


Figure 3.4 Effect of spraying ammonium sulfate (AMS) and urea at 4.4 lbs N acre<sup>-1</sup> weekly from May to October on silvery-thread moss (*Bryum argenteum* Hedw.) cover in an infested creeping bentgrass putting green at Colbert Hills Golf Club (Manhattan, KS). Means followed by the same letter on individual rating dates are not significantly different ( $P < 0.05$ ) according to Fisher's Protected LSD test.

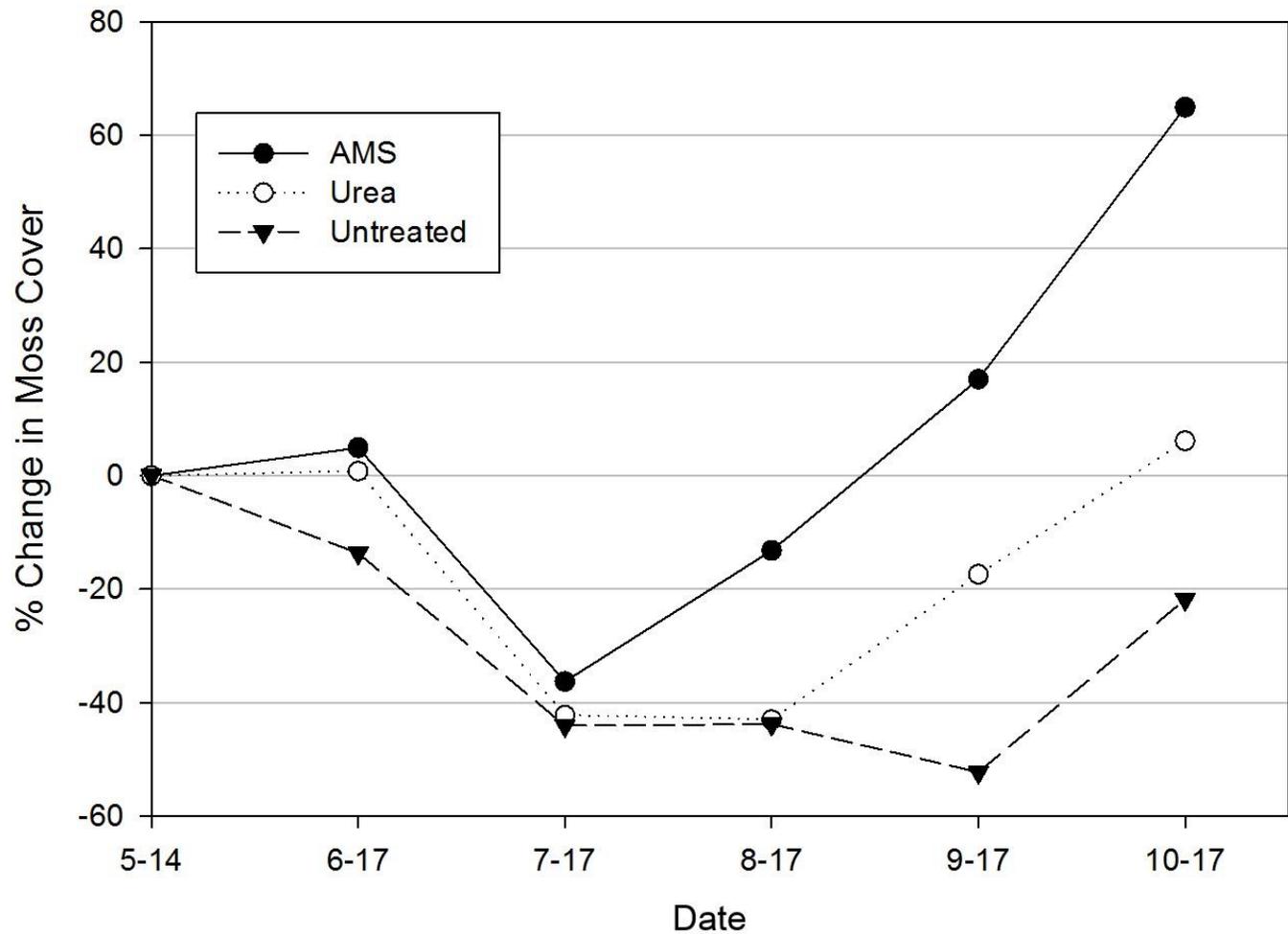


Figure 3.5 Effect of spraying ammonium sulfate (AMS) and urea at 4.4 lbs N acre<sup>-1</sup> weekly from May to October on silvery-thread moss (*Bryum argenteum* Hedw.) cover in an infested creeping bentgrass putting green at Rocky Ford Turfgrass Center in Manhattan, KS. There were no significant differences among treatments over the course of the study.



Figure 3.6 Effect of spraying ammonium sulfate (AMS) and urea to creeping bentgrass plots infested with silvery-thread moss (*Bryum argenteum* Hedw.); plots highlighted in black were treated with AMS or urea; plots highlighted in red are untreated. Light green areas are silvery-thread moss. Photo captured on 10/17/2014 at Rocky Ford Turfgrass Research Center in Manhattan, KS.

Table 3.1 ANOVA for percent silvery-thread moss (*Bryum argenteum* Hedw.) cover at various weeks after initial treatment (WAIT) when sprayed with two different nitrogen sources at differing spray volumes in the greenhouse: Run 1 and 2.

		<i>P</i> -value Associated with <i>F</i>							
		Moss Cover							
		WAIT							
Run 1	df	1	2	3	4	5	6	7	Dry Weight
N source	2	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Spray Volume	2	0.3906	0.0563	0.1636	0.0935	0.1432	0.7709	0.2633	0.1020
N × S	4	0.5098	0.0021	0.2695	0.5858	0.3893	0.4819	0.9525	0.2534
Run 2									
N source	2	0.0019	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Spray Volume	2	0.1442	0.9353	0.6005	0.6473	0.1875	0.2577	0.1105	0.6821
N × S	4	0.4652	0.2989	0.2507	0.5564	0.2840	0.3370	0.0150	0.4703

Table 3.2 ANOVA for nitrogen source effect on silvery-thread moss (*Bryum argenteum* Hedw.) cover at Rocky Ford Turfgrass Research Center and Colbert Hills Golf Course, Manhattan, KS.

<i>P</i> -value Associated with <i>F</i> for % Change in Moss Cover					
Location	Days After Initial Treatment				
	30	60	90	120	150
Rocky Ford	0.3554	0.4162	0.2932	0.1017	0.1251
Colbert Hills	0.1976	0.3376	0.1371	0.0391	0.0237

## **Chapter 4 - Dose Responses of Silvery-thread Moss (*Bryum argenteum*) to Carfentrazone**

### **Abstract**

Greenhouse dose response studies were conducted to determine the effectiveness of POST applications of carfentrazone on silvery-thread moss (STM) control. Carfentrazone was applied at 0, 14, 28, 56, and 112 g a.i. ha<sup>-1</sup> to pots containing established STM and creeping bentgrass. Percent gametophyte injury was visually estimated at 14, 28, 49, and 77 days after treatment (DAT). Shoot viability was determined by excising shoots from treated pots, and plating them in Petri dishes containing sand. The 28 and 49 DAT ED<sub>90</sub> (dose required to cause 90% gametophyte injury) were 26.8 and 54.3 g ai ha<sup>-1</sup>, respectively; both of these doses are substantially lower than the label rates for long- and short-term control, respectively. STM recovery did not occur until after 2 weeks after treatment (WAT), which indicates the label-stipulated application interval of 2 weeks is too short. All doses reduced the viability of shoots at 10 DAT compared to untreated STM; however, regrowth occurred in all Petri dishes by 17 DAT. As compared with label recommendations, this research suggests that lower carfentrazone rates, and longer intervals, may be effective for STM suppression in putting greens.

### **Introduction**

Quicksilver<sup>®</sup> (a.i. carfentrazone-ethyl), a phenyl triazolinone herbicide, is commonly used for selective control of silvery-thread moss (STM) in golf course putting greens in the United States. Carfentrazone-ethyl controls weeds by inhibiting protoporphyrinogen oxidase

(PPO), an essential enzyme in chlorophyll and heme biosynthesis (Senseman 2007). Inhibition of PPO ultimately results in the unregulated accumulation of protoporphyrin IX (Dayan et al. 1997). Protoporphyrin IX is a photodynamic pigment, and once energized by light, causes the formation of reactive oxygen species (Senseman 2007). These reactive oxygen species compromise the integrity of cell membranes via lipid peroxidation, and the leaky membranes result in rapid cell death (Duke et al. 1991). Because PPO is highly sensitive to phenyl triazolinone herbicides, use rates required to control weeds are very low (Cobb and Reade 2010).

Upon absorption, plants can rapidly hydrolyze carfentrazone-ethyl to its free acid derivative, carfentrazone; however, both are strong inhibitors of PPO (Dayan et al. 1997). Plants with selectivity, such as creeping bentgrass, are believed to further metabolize carfentrazone to nontoxic metabolites with the assistance of P-450 mono-oxygenases, while susceptible plants do so to a lesser extent (Dayan et al. 1997). The activity of carfentrazone is extremely fast with susceptible plants capable of showing herbicidal symptoms 2 hours after treatment (Thompson and Nissen 2000). Because of this rapid activity, several PPO inhibitors are now included as tank-mix partners with the slower acting synthetic auxin herbicides for broadleaf weed control in turfgrass.

In order to suppress STM, carfentrazone must reach the target site in lethal quantities to trigger the production of reactive oxygen species; a process which is highly dependent on the retention, absorption, and translocation of the herbicide (Cobb and Reade 2010). Unfortunately, the transport processes utilized by bryophytes, such as STM, are some of the most poorly understood areas in bryology (Glime 2007). Silvery-thread moss's high surface area-to-volume ratio creates a large amount of surface tension, enabling it to store and regulate the movement of extracellular water (Proctor et al. 2007). Therefore, the movement of carfentrazone is likely to be

highly dependent on the water status of STM. If translocation and absorption are reduced, then more herbicide will be required to obtain sufficient control. Additionally, Aro (1982) evaluated the composition of chlorophyll proteins and reported bryophytes had more chlorophyll associated with light-harvesting complexes and less with reaction center complexes compared to vascular plants, which may affect the lethal herbicide requirement compared to vascular plants.

Quicksilver has a supplemental label for STM control in bentgrass greens and tees. The label stipulates two STM control strategies: 1) For burndown and control of STM in bentgrass greens and tees, apply 112 g ai ha<sup>-1</sup>, followed by a second application two weeks later at the same rate; 2) For control over longer periods, applications may be repeated every two weeks at a rate no less than 33 g ai ha<sup>-1</sup>. Lastly, regardless of application strategy, no more than 448 g ai ha<sup>-1</sup> can be applied per year (Anonymous 2015). It is unclear why the application rates for STM control are relatively high (33 to 112 g ai ha<sup>-1</sup>) considering the label application rates for broadleaf weed control are 17 to 35 g ai ha<sup>-1</sup>. Several researchers have evaluated the efficacy of Quicksilver when applied at 111 g ai ha<sup>-1</sup> (Borst et al. 2010; Kennelly et al. 2010; Thompson et al. 2011); however, minimal research has evaluated lower use rates. In the only peer-reviewed report to date, Kennelly et al. (2010) applied carfentrazone-ethyl at 56 and 112 g ai ha<sup>-1</sup> to creeping bentgrass and reported no differences in STM control between the two rates throughout the study.

Additionally, once established, STM is likely to spread exclusively through the movement of asexual propagules, such as, protonema, gametophore fragments (shoots), and bulbils (Frey and Kürschner 2011; Hutsemekers et al. 2008; Raudenbush et al. 2015). Many of the cultural practices utilized by golf course superintendents, such as aerification, verticutting, grooming, and brushing are likely to dislodge and disseminate these propagules, especially

shoots. It seems plausible that an application of carfentrazone before implementation of the aforementioned cultural practices could reduce the viability of the dispersed STM shoots.

The possibility of obtaining STM control with lower carfentrazone rates is important information for superintendents from an environmental and budgetary standpoint. Therefore, the objective of this research was to determine the efficacy of POST applications of carfentrazone, at rates ranging from 1/8X to 2X (where X = the approximate label rate of 112 g ai ha<sup>-1</sup>), for control of STM.

## **Materials and Methods**

### ***Plant Material Maintenance and Preparation***

Growth chamber studies were conducted in 2014 and 2015 to determine the dose response of STM to carfentrazone. All studies were repeated. The STM population used in the studies was collected from a single colony (< 5 cm dia) in a research putting green at Rocky Ford Turfgrass Research Center in Manhattan, KS. The population was clonally propagated in the greenhouse for three months to obtain sufficient plant material. The propagated plant material was removed from the greenhouse and allowed to air-dry in the laboratory at 19°C for seven days. After seven days, ~5 g of dried STM gametophyte were placed in a coffee grinder (Krupps F20342, Millville, NJ) and ground for 6 to 7 s to thoroughly shred the plant material.

PVC containers (10 cm dia. by 23 cm deep) were filled with pea gravel to a depth of 4 cm, and then filled with 17 cm of moist sand conforming to USGA specifications (pH: 7.9; CEC: 2.75 meq 100 g<sup>-1</sup>) for a putting green rootzone. Containers (hereafter referred to as “pots”) were saturated and allowed to drain several times to encourage settling, and additional sand was added as needed to position the sand 2 cm from the top of the pot. After draining for 24 hr, each pot

was planted with 1.2 g of ground STM material. Plant material was evenly spread over the surface, and then immediately watered with a misting nozzle (Dramm 610SF, Manitowoc, WI) until nearly saturated. The misting nozzle was continuously moved while watering to prevent puddling of water at the soil surface. Puddling would have allowed the ground gametophyte material to float and consequently migrate towards the outer edge of the pots, reducing uniformity. Pots were watered three times daily for ten days, and then once daily for the remainder of the study. Silvery-thread moss gametophores began actively growing after 7 days. Pots were fertilized weekly throughout the study with a Hoagland 1:10 solution to provide  $3 \text{ kg N ha}^{-1} \text{ wk}^{-1}$ .

In putting greens, STM rhizoids attach to the crowns, roots, and shoots of desirable turfgrass species. Preliminary dose response studies on pots solely containing STM showed the importance of the rhizoid-turfgrass relationship, as treated gametophytes would begin to detach from the sand substrate, curling up at the pot edges. To prevent this from occurring, six 1.3 cm dia  $\times$  8 cm creeping bentgrass cv. 007 plugs were inserted through the STM gametophyte and into the sand substrate (Figure 4.1). The plugs and STM were allowed to acclimate for 30 d before any treatments were applied and plugs were trimmed twice weekly with scissors throughout the duration of the study. Plants were grown in the greenhouse for 90 d before treatments were applied. All plants used in the experiments had 100% cover and an average gametophore length of 2 cm. After 90 d, pots were transferred to the growth chamber (Convion E15, Winnipeg, Canada) and allowed to acclimate for 7 d. The growth chamber was set to a day/night temperature of  $20^{\circ}/17^{\circ}\text{C}$  (HOBO Pro V2, Onset Computer Corporation, Bourne, MA) with a 16 hr photoperiod emitting  $690 \mu\text{molm}^{-2} \text{ s}^{-1}$  of photosynthetically active radiation (AccuPar LP-80, Decagon Devices, Pullman, WA) at 30 cm above the pots.

### *Gametophyte Injury and Shoot Viability*

Carfentrazone was applied at rates of 0, 14, 28, 56, 112, and 224 g ai ha<sup>-1</sup>. Herbicides were applied using a moving-track spray chamber (Devries Manufacturing, Hollandale, MN) affixed with a TeeJet XR8002EVS nozzle, operating at 220 kPa to deliver a spray volume of 220 L ha<sup>-1</sup>. All treatments were applied with a nonionic surfactant (0.25% v/v). Percent gametophyte injury was visually estimated weekly for eleven weeks. Plants with necrotic gametophore tips were considered dead, while green tips were considered healthy. At 77 DAT, STM gametophytes were harvested and fresh weight was recorded. Harvested plant material was dried at 70°C for 2 d and dry weight was recorded.

Shoot viability was also determined by harvesting individual shoots (gametophores) directly from the pots described above. At 3 days after treatment (DAT), approximately 40 total shoots were harvested from four random areas of each pot using fine point forceps. Harvested shoots were immediately submerged in 1.5 mL centrifuge tubes containing double-distilled water. Eight randomly selected shoots from each centrifuge tube were placed in 51 mm petri dishes filled with sand (previously described) which had been autoclaved for 20 min at 120°C at a pressure of 98 kPa; sand was added to the dishes to a depth of ~ 5 mm. Shoots were arranged in two parallel rows, each with four shoots, across the middle of the dishes. Double distilled water was pipetted over both rows of shoots until a light film of water appeared at the soil surface and then lids were placed on the dishes. Dishes were placed in the previously described growth chambers and double distilled water was pipetted into dishes every 4 d to maintain a film of water at the surface. At 7 and 14 days after plating (DAP) the viability of individual shoots was determined by counting the number of erect shoots with > 5 leaves and by visually estimating the percent of a shoot covered by protonema. Structures were identified and counted using a dissecting microscope (SMZ645, Nikon Co., Tokyo, Japan).

### ***Experimental Design and Analysis***

A completely randomized design with four replicates and a one-way treatment structure was used to evaluate the effect of carfentrazone dose on several STM growth parameters. Percent gametophyte injury, gametophyte fresh and dry weight, shoot viability counts, and percent protonema were subjected to ANOVA using the GLM procedure in SAS. Experimental run was included in the model, and data were combined when a significant run  $\times$  dose interaction ( $P < 0.05$ ) was not observed. Means were separated using Fisher's protected LSD test ( $P < 0.05$ ). Percent gametophyte injury at 28 and 49 DAT were further analyzed using nonlinear regression. A three parameter log-logistic model was fit to the data because it had the lowest bias-corrected Akaike information criterion. The log-logistic model was used to calculate the dose of carfentrazone-ethyl required to cause 50 and 90% gametophyte injury at 28 and 49 DAT. As described by Kniss and Lyon (2011), the lower limit of the log-logistic model is constrained to zero, so the equation takes the form of:

$$Y = d / \{1 + \exp[b(\log x - \log e)]\} \quad [1]$$

where  $Y$  is percent gametophyte injury;  $d$  is the upper asymptote;  $b$  is the slope around  $e$ ;  $x$  is the rate of carfentrazone; and  $e$  is the rate of carfentrazone required to cause 50% of the maximum response.  $ED_{50}$  and  $ED_{90}$  values were estimated using the sensitivity index function in the *drc* package (Knezevic et al. 2007) in R (R Foundation for Statistical Computing, Vienna, Austria, 2014).

### **Results and Discussion**

A dose  $\times$  run interaction was not present for gametophyte fresh- and dry-weight, shoot viability, and percent protonema measurements (Tables 4.1 and 4.2); therefore, data were

combined for those parameters. Additionally, there was no dose  $\times$  run interaction for gametophyte injury data at 28 and 49 DAT, but there was an interaction at 14 and 77 DAT (Table 4.1). Therefore, we combined data for the 28 and 49 DAT rating dates, and discussion of gametophyte injury will be focused on those dates, for the following reasons: Close inspection of the interaction plots (not shown) revealed that the interactions at 14 DAT was due to very slight “injury” (<10%) in the untreated STM from run 2, while all higher doses in run 2 caused similar, but slightly less, injury to STM than in run 1; thus, the interaction was not judged to be a meaningful one. More importantly, the 28 and 49 DAT rating dates were the most definitive in making determinations of gametophyte injury. For example, we had anticipated that 14 g ai ha<sup>-1</sup> (the 1/8X rate) would cause considerably less injury compared to higher doses at 14 DAT, but all doses caused > 85% injury (Figure 4.1); and insights gained from the 77 DAT data were not substantially different those obtained from the 49 DAT data. The main difference was that overall injury levels at 77 DAT were lower for all doses, as the STM continued to recover.

At 28 DAT, 14 g ai ha<sup>-1</sup> of carfentrazone caused 83% injury to STM gametophytes, which was significantly less than the > 97% injury caused by the 56, 112, and 224 g ai ha<sup>-1</sup> doses (Figure 4.2). The estimated ED<sub>90</sub> value for gametophyte injury at 28 DAT was 26.8 g ai ha<sup>-1</sup>. At 49 DAT some recovery had occurred, and the 14 g ai ha<sup>-1</sup> had only 50% gametophyte injury (Figures 4.2 and 4.3). The 112 and 224 g ai ha<sup>-1</sup> doses had > 90% injury, which was significantly more than with 14 and 28 g ai ha<sup>-1</sup>, but not different than with 56 g ai ha<sup>-1</sup>. The ED<sub>90</sub> value at 49 DAT was 54.3 g ai ha<sup>-1</sup>, which was a 2-fold increase compared to the ED<sub>90</sub> value at 28 DAT. Therefore, superintendents spraying multiple applications at intervals up to 4 weeks should expect to see adequate control with 26.8 g ai ha<sup>-1</sup>; while superintendents who make less frequent applications would benefit from the higher rate of 54.3 g ai ha<sup>-1</sup>. The programs described above

differ somewhat from those currently on the Quicksilver label. The experimental 26.8 g ai ha<sup>-1</sup> is similar to the 33 g rate prescribed for “control over longer periods”; however, the label stipulates applying at 2 week intervals, whereas we did not observe recovery until after 2 WAT. The 54.3 g ai ha<sup>-1</sup> is a 50% reduction from the higher 112 g label rate of Quicksilver. In this study, no differences in injury between the 56 and 112 g dose were observed; similar to the results presented by Kennelly et al. (2010).

Silvery-thread moss gametophyte growth was also affected by carfentrazone dose (Table 4.1). Untreated STM had higher fresh- and dry-biomass accumulation compared to those treated with carfentrazone (Figure 4.4). Notably, there were no differences in biomass accumulation of STM among carfentrazone doses tested (Figure 4.4); while several doses caused > 90% visual injury, that injury was not accompanied by proportionate decreases in fresh- and dry-weight. This result demonstrates a remarkable ability of STM gametophytes to resist decay after being injured by carfentrazone. After being treated with herbicides, most vascular plant carcasses eventually lose their turgidity, collapse, and begin to decompose. However, bryophytes have a slower decomposition rate compared to vascular plants (Scheffer et al. 2001). In fact, many bryophytes actively produce microbial inhibitors to decrease the rate of decomposition (Glime 2007). This slower rate of decomposition is a key factor in understanding why applying carfentrazone alone for STM control may be unsuccessful: Bentgrass stolons and tillers may have difficulty penetrating the dense STM gametophyte, even after it has been injured by carfentrazone. Therefore, following carfentrazone application, superintendents should consider implementing hollow-tine aerification or verticutting to create available sites within the injured gametophyte for desirable turfgrass species to occupy. (Raudenbush and Keeley 2015). For small infestations, mechanical removal with a knife or cup cutter may be more practical and less

disruptive to play. Ultimately, STM's slower decomposition rate presents challenges for management; therefore, practices to increase the rate of STM decomposition are possible avenues for future research.

Once established, STM can spread through dislodged shoots, increasing the size of an infestation (Raudenbush et al 2015). If the viability of dislodged shoots could be reduced by carfentrazone, then superintendents could make an application before implementing practices that are likely to dislodge shoots (e.g., aerification, verticutting, grooming, brushing, etc.), to reduce the spread and establishment of new colonies. This research showed that carfentrazone did affect the viability of individual shoots (Table 4.2 and Figure 4.5). At 7 DAP (corresponding to 10 DAT), petri dishes containing shoots harvested from untreated STM had a mean count of 7.8 shoots with > 5 leaves, while shoots harvested from STM receiving any carfentrazone dose had none (data not shown). The erect shoots in untreated pots were not new leaf primordia, rather, the existing gametophore tip produced rhizoids and exhibited positive phototropism (Figure 4.6). Secondly, a mean shoot count of 7.8 means that almost every plated shoot produced rhizoids and began to establish in 7 d, which may have important implications for managing the spread of STM. Furthermore, while no carfentrazone-treated STM had erect shoots with >5 leaves at 7 DAP, nearly all of the excised shoots (from all carfentrazone doses) were producing new leaf primordia and protonema (Figure 4.6b).

By 14 DAP, all petri dishes contained a large number of erect shoots; however, the 112 and 224 g ai ha<sup>-1</sup> rates of carfentrazone reduced the number of shoots compared to the untreated (Figure 4.5). This raises an interesting question: Why did the shoots in petri dishes exhibit such prolific and rapid regrowth, but the intact gametophytes took  $\geq 28$  d to exhibit recovery? Two scenarios seem plausible: First, although it was not directly measured, relative humidity in the

covered petri dishes is likely near 100%, while the relative humidity in the growth chamber was ~60%. Thus, higher humidity in the petri dishes may have created a favorable environment for protonema production, and consequently, new leaf primordia. Second, an STM gametophyte is very dense, consequently, only the uppermost leaves receive exposure to light. Once plated in the petri dishes, the entire shoot was exposed to light, which may have triggered the production of new leaf primordia. In any case, eight individual shoots were capable of producing > 60 new plants at the highest dose in our study (Figure 4.5), which reinforces the importance of taking action in the early stages of an STM infestation, considering a colony 5 cm in diameter can contain thousands of individual shoots.

In summary, the ED<sub>50</sub> and ED<sub>90</sub> values suggest that lower doses of carfentrazone are effective at injuring STM (Figure 4.2). Additionally, the current Quicksilver label stipulates spraying carfentrazone at two-week intervals. In our research, no regrowth was observed until after 2 WAT. Superintendents should closely monitor gametophyte colonies following an application of carfentrazone. This herbicide inhibits an enzyme involved in chlorophyll production; therefore, if STM has no green, healthy tissue, then minimal sites will be available for further injury. Superintendents should consider extending the application interval to three or four weeks, by which time some regrowth will likely have occurred.

The ability of carfentrazone to reduce the viability of fragmented shoots, makes it a potentially valuable tool for use in conjunction with the cultivation practices commonly used on putting greens. Superintendents managing STM infestations should consider making an application of carfentrazone about one week before the implementation of canopy-invasive practices, such as core-aerification, verticutting, and brushing. Because the carfentrazone-treated

shoots in our study showed the ability to regenerate, a follow-up application two weeks after canopy-invasive practices will help control any dispersed plant material.

It is important to acknowledge that plants are typically easier to control in a greenhouse or growth chamber, than in the field. However, even the lowest rates used in our study caused significant injury to STM, which certainly warrants testing of lower rates in the field. The STM injury caused at 56 g ai ha<sup>-1</sup> (1/2X) was similar to that at 112 g ai ha<sup>-1</sup> in this study, and after 28 days, the 28 g ai ha<sup>-1</sup> (1/4X) showed >90% injury to STM. These rates should be evaluated in field studies, using 3 to 4-week application intervals. Lastly, carfentrazone is an extremely valuable tool for reducing the performance of STM; however, the ability of this plant to resist decay is perhaps its best weedy attribute. Future research to determine practices that encourage the breakdown and decomposition of treated gametophytes would be valuable.

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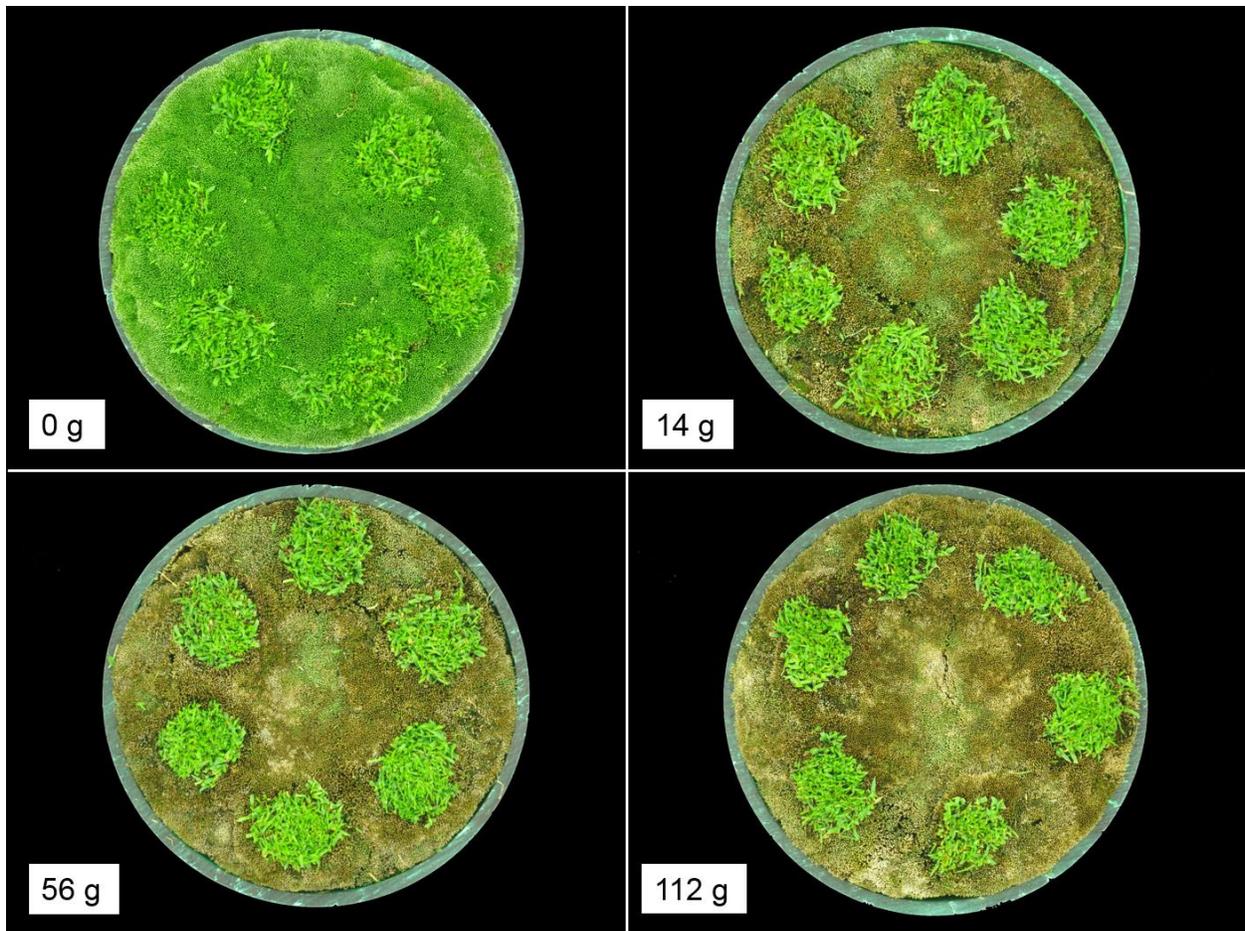


Figure 4.1 Lightbox images showing silvery-thread moss (*Bryum argenteum* Hedw.)

gametophyte injury at 14 days after treatment from an application of carfentrazone. The six small, circular green patches are creeping bentgrass plugs. Doses, expressed in g a.i. ha<sup>-1</sup>, are shown in bottom-left corners of images. The label rate for STM control in creeping bentgrass putting greens is 112 g a.i. ha<sup>-1</sup>.

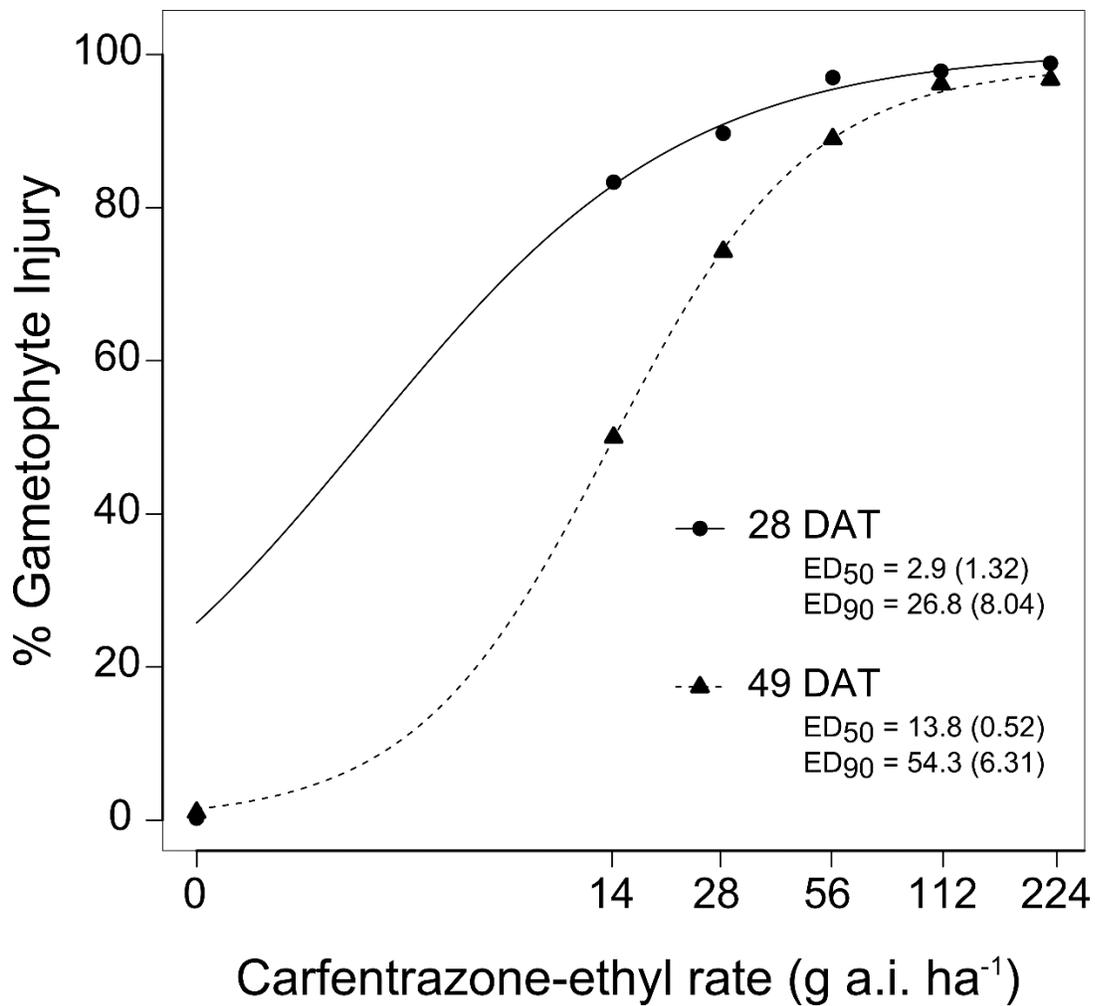


Figure 4.2 Percent silvery-thread moss (*Bryum argenteum* Hedw.) gametophyte injury, and ED<sub>50</sub> and ED<sub>90</sub> values, as influenced by carfentrazone application rate at 28 and 49 days after treatment (DAT). Values within parenthesis are the standard error ( $\pm$ ) for each ED value as predicted by the log-logistic model. The label rate for STM control in creeping bentgrass putting greens is 112 g a.i. ha<sup>-1</sup>.

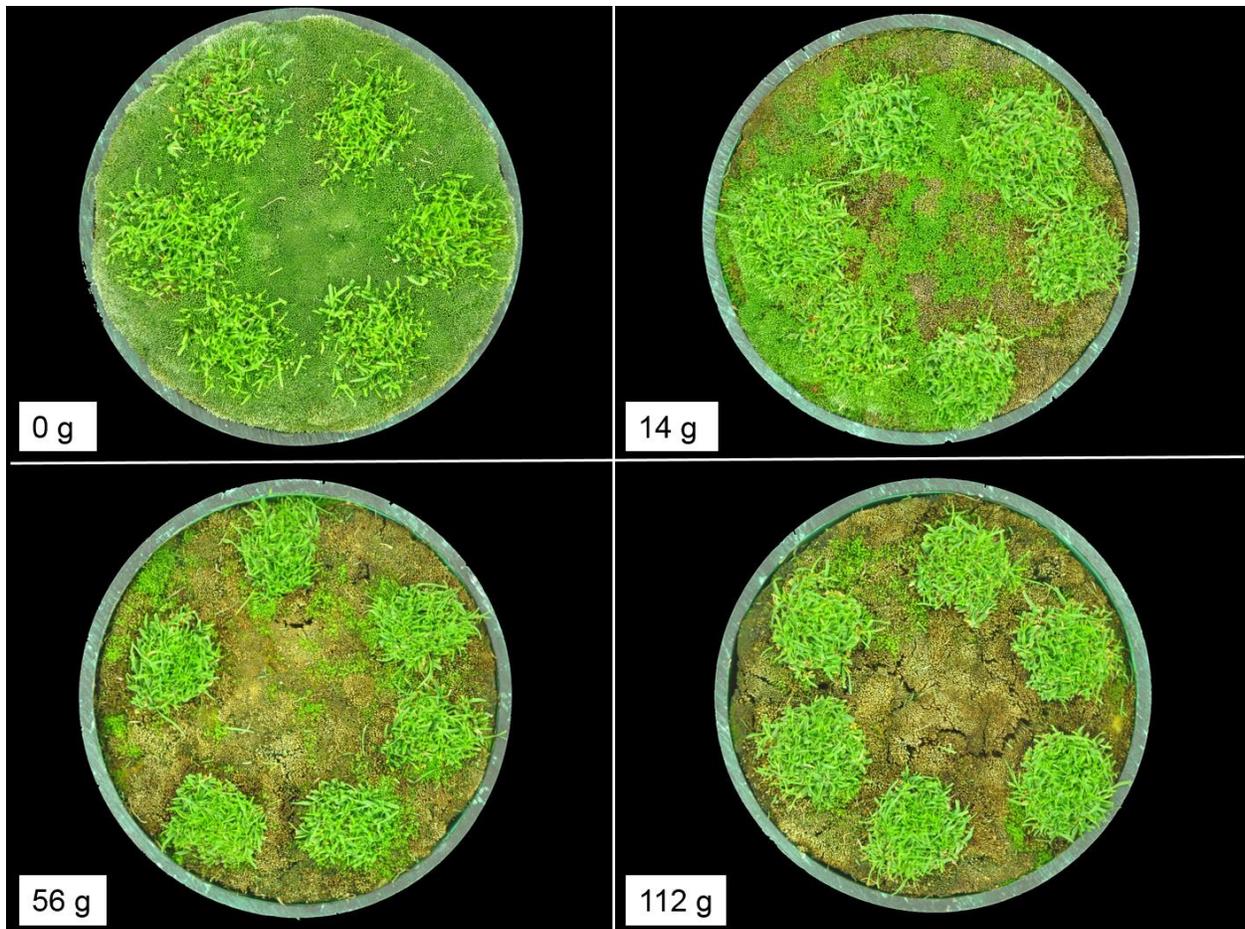


Figure 4.3 Lightbox images showing silvery-thread moss (*Bryum argenteum* Hedw.)

gametophyte injury at 49 days after treatment from an application of carfentrazone-ethyl.

Doses, expressed in g a.i. ha<sup>-1</sup>, are shown in bottom-left corners of images. The label rate for STM control in creeping bentgrass putting greens is 112 g a.i. ha<sup>-1</sup>.

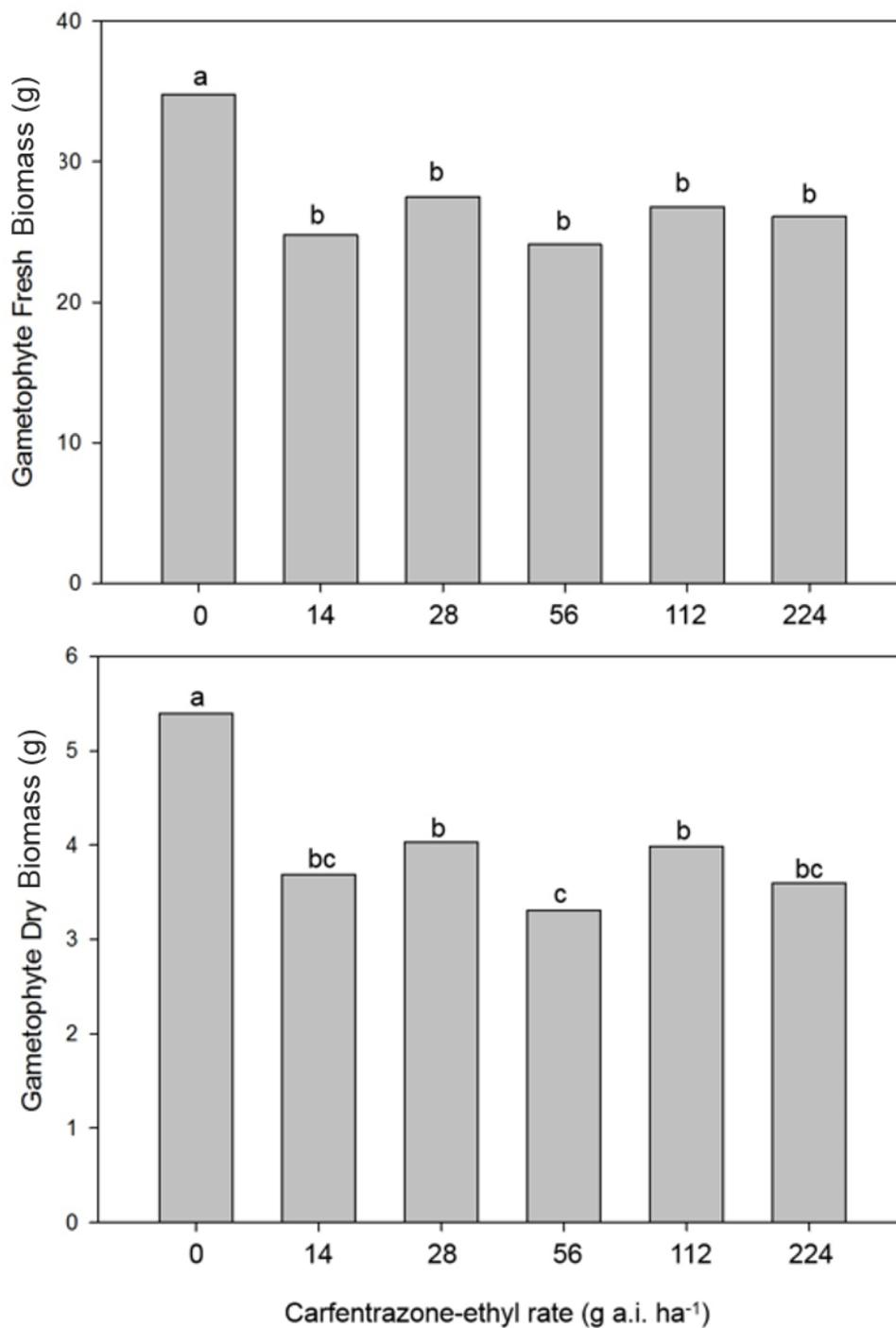


Figure 4.4 Effect of carfentrazone-ethyl on gametophyte fresh- and dry-biomass harvested at 77 days after treatment. Within each graph, treatments with the same letter above the bar are not significantly different ( $P < 0.05$ ) according to Fisher's protected LSD test.

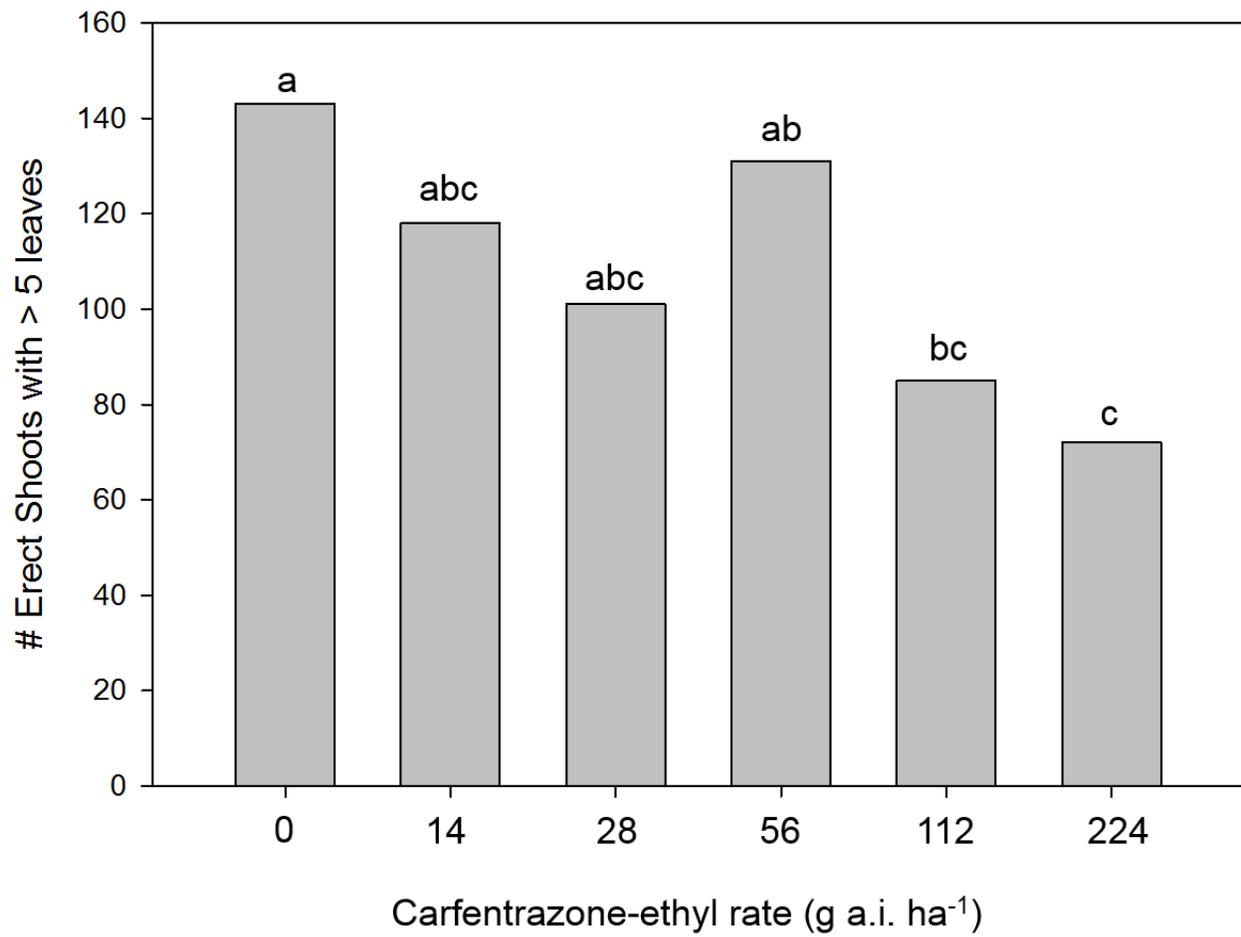


Figure 4.5 Viability of eight plated silvery-thread moss (*Bryum argenteum* Hedw.) shoots 17 d after being harvested from pots treated with differing rates of carfentrazone-ethyl.

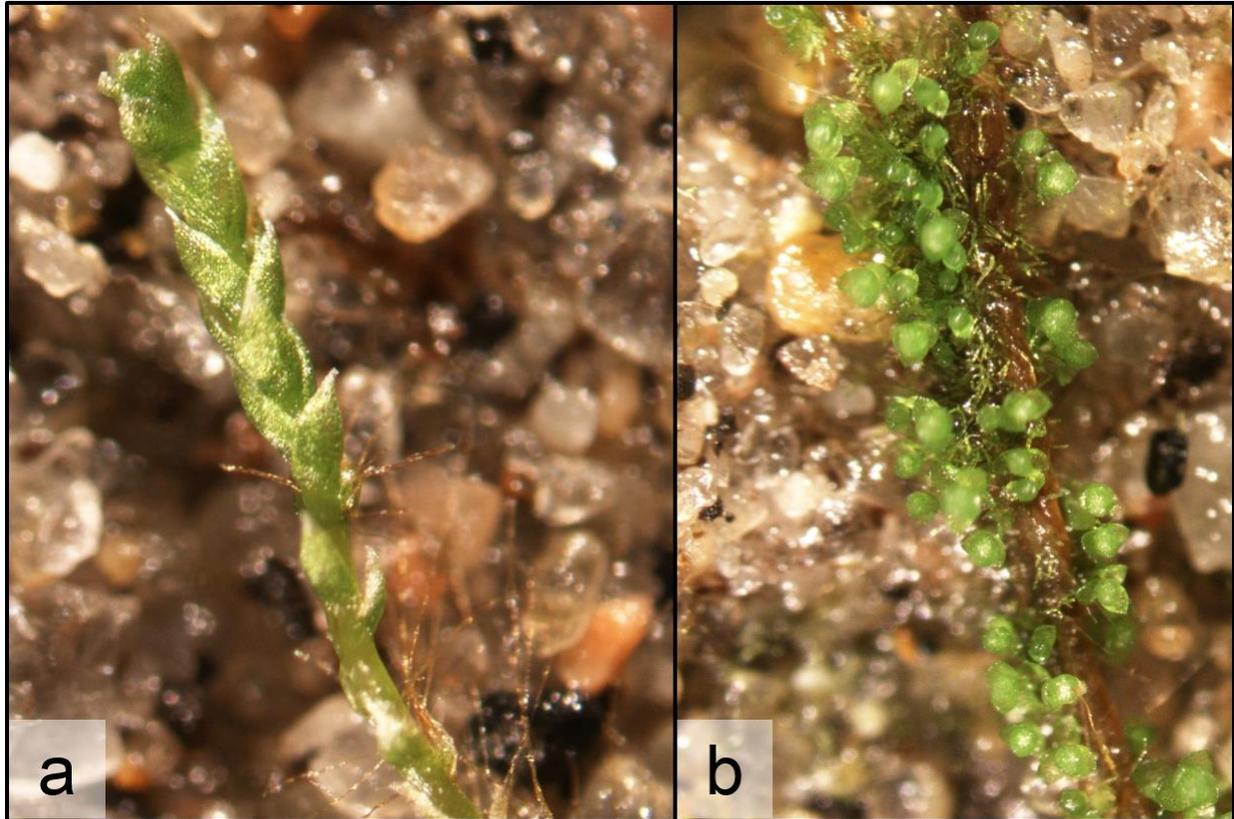


Figure 4.6 Silvery-thread moss (STM; *Bryum argenteum* Hedw.) shoots excised from carfentrazone-treated STM in a growth chamber: a) Untreated shoot producing rhizoids and exhibiting positive phototropism; b) shoot excised from STM treated with carfentrazone at 56 g ai ha<sup>-1</sup> producing protonema and new leaf primordia.

Table 4.1 ANOVA for percent gametophyte injury at various days after treatment (DAT) and gametophyte fresh- and dry-weights at harvest when sprayed with differing doses (0, 14, 28, 56, 112<sup>†</sup>, 224 g a.i. ha<sup>-1</sup>) of carfentrazone.

		<i>P</i> -value Associated with <i>F</i>					
Source	df	% Gametophyte Injury				Weight (g)	
		14 DAT	28 DAT	49 DAT	77 DAT	Fresh	Dry
Dose	5	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Run	1	0.0086	0.3651	0.1291	0.001	<.0001	<.0001
D × R	5	0.0001	0.7947	0.8386	0.0071	0.3553	0.2336

<sup>†</sup> Highest label rate for silvery-thread moss control in creeping bentgrass putting greens.

Table 4.2 ANOVA for total number of erect shoots with > 5 leaves, and percent of shoot covered with protonema when harvested from STM treated with differing doses (0, 14, 28, 56, 112<sup>†</sup>, 224 g a.i. ha<sup>-1</sup>) of carfentrazone at 7 and 14 days after treatment (DAT).

		<i>P</i> -value Associated with <i>F</i>			
		Total Shoots		% Protonema	
Source	df	7 DAT	14 DAT	7 DAT	14 DAT
Dose	5	<.0001	0.0340	0.0464	0.5111
Run	1	0.1519	0.0207	0.0034	0.2520
D × R	5	0.4863	0.7088	0.5816	0.8440

<sup>†</sup> Highest label rate for silvery-thread moss control in creeping bentgrass putting greens.

## **Chapter 5 - Effect of Irrigation Water pH and Acidifying-Source on Silvery-Thread Moss (*Bryum argenteum* Hedw.) Establishment**

### **Abstract**

Many bryophytes are intolerant of osmotic stresses; nevertheless, silvery-thread moss (STM) often infests golf course greens irrigated with water containing a moderate amount of sodium and/or bicarbonates. Because of the poor quality of such water, irrigation systems may be retrofitted with acid injection systems, which could potentially be encouraging the growth of STM. Therefore, the objective of this study was to determine if altering the pH of sodic irrigation water using either sulfuric- or hydrochloric acid affects the growth of STM. Secondarily, if there was an effect on STM growth, we sought to understand whether that effect was due to differences in nutrient uptake or availability under the different water pH's and/or acidifying sources. Phosphate buffer solutions (0.01 M, pH=9) were titrated with each acid to obtain pH's 5, 6, 7, and 8. The buffer solution served as the control. Irrigation solutions were applied daily to pots containing a sand substrate and nascent STM for 28 d. Percent STM cover was determined weekly using digital image analysis. Tissue and soil analysis was conducted after 28 d. Acid-source had no effect on moss cover, but pH significantly affected STM cover. Generally, pots irrigated with pH's 5 and 6 had six- to seven-fold increases in STM cover compared to pH's 7 and 8. Additionally, the gametophyte tissue of pots treated with pH 5 contained twice as much potassium compared to pots irrigated with pH 9.

## Introduction

Several bryophytes, such as silvery-thread moss (STM) are ectohydric, and capable of absorbing water and dissolved nutrients across the entire surface of the gametophyte (Chopra and Kumra, 1988). Because of this unique morphology, STM cannot extract water and nutrients from deep within the soil profile like vascular plants; rather, water is supplied from irrigation, rainfall, or dew. Vascular plants differ from STM because they rely on symplastic or apoplastic pathways to transport water and nutrients; however, STM can also transport water and nutrients via external capillary action along leaf surfaces (Proctor, 2000). The external movement of water is much more rapid than internal movement in STM (Glime, 2007) and a considerable amount of water is held in the capillary spaces of the gametophyte (Chopra and Kumra, 1988; Proctor, 1979). Because the majority of STM's water is stored externally, its water status is highly influenced by the surrounding environment. During droughty periods, evaporation exhausts external capillary water, and STM cells quickly dry out and cease metabolism (Proctor et al., 2007). However, metabolism rapidly resumes when water is resupplied, so STM typically exists in a desiccated or fully turgid state.

Water is arguably the most limiting factor of STM growth, which may explain its competitiveness in golf course putting greens. Many putting greens are constructed using a sand substrate, allowing for adequate drainage while resisting compaction; consequently, they often require irrigation two or more times weekly during the summer months. Because of this high irrigation frequency, STM is likely supplied enough water to remain turgid and actively metabolizing throughout most of the growing season. Additionally, putting greens are typically mown  $\geq 6$  times per week in the early morning; therefore, STM can absorb the dew or guttation water that is dislodged from leaf blades during mowing. Lastly, the microclimate created by the turfgrass canopy helps STM reduce and regulate water loss: In a putting green, STM is

submersed in the laminar boundary layer created by the transpiring turfgrass leaves, which helps reduce the amount of water lost via gas exchange. Several other factors contribute to the success of STM in putting greens (Raudenbush et al., 2015), but a plentiful supply of water is perhaps the most important.

The STM gametophyte acts similar to a sponge, wherein external water is directly absorbed by the gametophyte and stored in the capillary spaces between shoots. Because of this unique adaption, STM may have increased sensitivity to changes in the chemical composition of supplied external water compared to vascular plants, which are somewhat protected by the buffering capacity of the soil (Brady and Weil, 2002). The potential sensitivity of STM to changes in water quality is of interest because golf course irrigation sources are frequently less than ideal; specifically, they may be of high pH, and contain excessive sodium and/or bicarbonate (Whitlark, 2010). Consequently, irrigation pump houses are often retrofitted with acid injection systems to acidify the water and combat these potentially harmful salts; however, scientific research regarding their effectiveness is limited (Christians, 1999). Bryophytes are reported to be relatively intolerant of osmotic stress (Glime, 2007); nevertheless, STM is often a successful invader of putting greens irrigated with water containing a considerable amount of sodium and/or bicarbonates. This incongruity raises questions about how acid injection systems could be influencing the growth of STM.

Sulfuric acid is arguably the most cost effective acidifying source, and is commonly used to reduce irrigation water pH to ~6.5 (Whitlark, 2010), but this process may be increasing the competitiveness of STM. Little is known concerning the effect of pH of the irrigation water on STM, but Raudenbush and Keeley (2014) fertilized STM with foliar applications of either ammonium sulfate (AMS) or urea, and reported AMS increased STM growth more than urea.

They speculated that the positive effect on STM growth with AMS may have been due to a pH reduction of the extracellular water retained by the gametophyte, or to the additional sulfur supplied by AMS.

When sulfuric acid is injected into irrigation water, it both reduces pH of the irrigation water, and supplies sulfate. If either of these effects increases STM growth, then golf courses utilizing sulfuric acid injection systems could be predisposed to STM infestations. If sulfate, but not lower irrigation water pH, increases STM growth, then non-sulfurous acids such as hydrochloric acid could be used to acidify irrigation water without increasing STM growth. Currently, no research is available regarding the effects of irrigation water pH or acidifying source on STM. Therefore, the objectives of this study were to: 1) determine if altering the pH of irrigation water affects the growth of STM, and 2) determine if sulfuric acid increases STM growth compared to a non-sulfurous acid such as hydrochloric acid. Secondly, if there was an effect on STM growth, we sought to understand whether that effect was due to differences in nutrient uptake under the different water pH's and/or acidifying sources.

## **Materials and Methods**

A greenhouse study was conducted in the Throckmorton Plant Science Center at Kansas State University, Manhattan, KS to determine the effects of irrigation water pH and acidifying source on STM growth and nutrient uptake. Two experimental runs of the study were conducted, with the second run starting one week after the first. The greenhouse was maintained at a day/night temperature of 20°/15° C (HOBO Pro V2, Onset Computer Corporation, Bourne, MA) throughout the duration of the study. The STM population used in the experiment was a colony

originally collected from a research green at Rocky Ford Turfgrass Research Center in Manhattan, KS. The population was clonally propagated in the greenhouse for several months prior to trial initiation to obtain sufficient plant material. The propagated plant material was removed from the greenhouse and allowed to air-dry in the laboratory at 20° C for 7 days. After 7 days, 5 g of dried plant material were placed in a coffee grinder (Krupps F20342, Millville, NJ) and ground for ~6 s, until the plant material was sufficiently shredded.

PVC containers (10 cm dia. by 22 cm deep) were filled with pea gravel to a height of 5 cm, and then filled with 16 cm of moist sand conforming to USGA specifications (pH: 7.9; CEC: 2.75 meq 100 g<sup>-1</sup>) for a putting green rootzone. Containers (hereafter referred to as pots) were saturated and allowed to drain several times to encourage settling, and additional sand was added as needed to position the sand 2 cm from the top of the pot. After pots drained for 24 h, each pot was planted with 1.2 g of ground STM material. Plant material was evenly spread over each individual pot and immediately watered with a misting nozzle (Dramm 610SF, Manitowoc, WI) until nearly saturated. The misting nozzle was continuously moved while watering the pots to prevent puddling of water at the soil surface. Puddling would have allowed the ground gametophyte material to float and consequently migrate towards the outer edge of the pots, reducing uniformity. Pots were watered twice a day for 14 days. Silvery-thread moss gametophores began actively growing after 10 days. For each run of the experiment, 20% more pots were planted than were needed to complete the experiment; the pots with the most homogeneous STM cover were selected for each run to improve homogeneity of experimental units. Pots had a mean STM cover of 8% at trial initiation in run 1, and 12% in run 2.

A two-way factorial treatment structure was used to evaluate the effect of irrigation pH (4 levels) and acidifying source (2 levels) on STM growth and nutrient uptake. Irrigation solutions

were prepared in 20 L plastic buckets (Model # 05GLHD2, Home Depot, Atlanta, GA) in the laboratory using double-distilled water with a final solution volume of 8 L. All solutions were prepared from a 0.01 M phosphate buffer comprised of reagent grade  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  and  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$  at concentrations of 0.0002 and 0.0098 M, respectively. The previous salts were added to buckets containing 8 L of double-distilled water and agitated with a stirring plate and octagonal magnet until salts were completely dissolved. The stock 0.01 M phosphate buffer solution had a pH of 9. A pH probe (AB150, Accumet) was submerged into the agitating solution and HCl or  $\text{H}_2\text{SO}_4$  was titrated into the buffered solutions to obtain pH's of 5, 6, 7, and 8 for each acid. The prepared irrigation solutions were applied to the pots containing nascent STM every day for four weeks using a 100 mL Griffin beaker. Each pot received 40 mL of solution, which was slowly poured to prevent puddling and ensure uniform distribution. Because the solutions had moderately high EC values ( $\sim 1.8 \text{ ds m}^{-1}$ ), pots were flushed once per week with 100 mL of distilled water to mimic a rainfall or flushing event. Additionally, pots were fertilized weekly with solubilized urea and dibasic potassium phosphate to supply 4.9 kg N, 0.42 kg P and 2.02 kg K  $\text{ha}^{-1}$  per week. Treatments were administered for 28 d.

Percent STM cover was determined weekly using overhead digital images, which were batch-processed using the methods described by Richardson et al. (2001). Images were obtained using a Nikon D3000 (Nikon Co., Tokyo, Japan) digital single-lens reflex camera affixed to a custom-built lightbox ( $0.5 \times 0.7 \times 0.6 \text{ m}$ ) containing four 6500 K-temperature fluorescent light bulbs (model ESL23TM/D, Feit Electric Co., Pico Rivera, CA). The camera utilized a shutter speed of 1/125 s, aperture of F5.6, ISO 800, and focal length of 50 mm. Images were saved as JPEG format because light conditions were uniform and well-controlled throughout the experiment. Percent cover was extracted from images using SigmaScan Pro (Version 5.0, SPSS,

Chicago, IL). The program was able to selectively identify individual STM gametophores utilizing a hue range of 60-75 and a saturation range of 50-100. “Difference in percent cover from control” was determined by comparing STM cover under each irrigation treatment to STM cover in pots receiving only the stock 0.01 M phosphate buffer solution (pH 9). The calculation was as follows: Difference in % cover from control = (% cover of treatment pot - % cover of control pot within respective replicate). A negative value indicates a treatment had lower percent cover compared to the control.

At 4 weeks after initial treatment (WAIT), STM gametophytes were carefully discarded and the top 2.5 cm of soil were removed from each pot and sent to the soil testing lab for analysis. All samples were analyzed using the methods described in “Recommended Chemical Soil Test Procedures for the North Central Region” (Anonymous, 1998). Samples were analyzed for pH, available phosphorus, potassium, calcium, magnesium, sodium, iron, manganese, zinc, ammonium, and nitrate. Soil testing procedures and extractants are outlined in Table 5.1.

An ancillary study was conducted to determine nutrient uptake by sampling the tissue nutrient content of STM irrigated with water having pH 5, 7, or 9. We originally intended to perform this nutrient analysis using gametophyte tissue from the larger, percent cover study mentioned above; however, there was insufficient STM tissue for analysis under several of the treatments. Therefore, it was necessary to administer the irrigation treatments to established gametophytes. To accomplish this, STM was planted in pots in the greenhouse using the same procedures previously described. The pots were misted twice per day using tap water and fertilized weekly with a 1:10 Hoagland’s solution for two months. At two months, the gametophyte was dense and had gametophores ~1 cm in length. Three irrigation treatments (pH 5, 7, 9) were prepared and applied daily, as previously described, for 14 d to the two-month-old

gametophytes; pH's 5 and 7 were obtained from HCl. After 14 d, a curved iris scissor was used to harvest the gametophore tips; approximately 4 g of fresh gametophore material was harvested from each pot (Figure 5.1). Tissue was dried at 70° C for 48 h and submitted to the Kansas State soil testing laboratory for analysis of P, K, NH<sub>4</sub>, NO<sub>3</sub>, Ca, Fe, and Mg content using nitric-perchloric acid digest and combustion methods.

A completely randomized design with three replicates was used in run 1, and four replicates in run 2, to evaluate the water pH/acid source treatments in the cover study. A repeated measures analysis was conducted for difference in percent STM cover from control in the MIXED procedure of SAS, and experimental run was treated as a random effect. The error structure was fit with an autoregressive heterogeneous model, as it had the lowest Akaike and Bayesian information criterion values. Means within each WAIT were separated using Fisher's protected LSD test ( $P < 0.05$ ).

For the tissue analysis study, a completely randomized design with four replicates and a one-way treatment structure was used, in which irrigation water pH was the independent variable. Tissue and soil test data were analyzed using the GLM procedure in SAS and means separated using Fisher's protected LSD test ( $P < 0.05$ ).

## **Results and Discussion**

Silvery-thread moss cover was highly affected by pH of the irrigation water, but acid source had no effect (Table 5.2). It was previously hypothesized that foliarly applied AMS may have enhanced the growth of STM by the addition of sulfate. If this were true, we would expect acid-source to be significant in the ANOVA. The fact that it was not is, perhaps, a best-case scenario for superintendents, considering sulfuric acid is one of the most economical

amendments for reducing irrigation water pH (Whitlark, 2010). The effect of pH on STM growth changed throughout the duration of the study, causing a pH × WAT interaction; therefore, means for each rating date are presented separately (Figure 5.2).

The effect of pH on STM cover was pronounced at every rating date, with pH's 5 and 6 having higher coverage than pH's 7 and 8 (Figure 5.2). Trends were similar among treatments at 2, 3, and 4 WAT; however, the pH × WAT interaction likely stems from the results at 1 WAT (Figures 5.2 and 5.3). After 1 WAT, pH's 5 and 6 were not different but always had higher coverage compared to pH's 7 and 8 (Figures 5.2 and 5.4). By contrast, at 1 WAT, pH 6 had more STM cover than pH 5, but both had higher cover than pH's 7 and 8.

The slower growth of STM in pots irrigated with a pH  $\geq 7$  was surprising, considering many bryologists culture STM *in vitro* using distilled water, which typically has a pH  $\sim 7$ . In this study, we used a buffer comprised of mono- and di-basic sodium phosphate to ensure the pH of the solutions remained stable throughout the experiment, while mimicking a situation in which an acid injection system may be used on a golf course; consequently, solutions contained a moderately high amount of sodium, which is reflected in an average tested EC value of 1.8 ds m<sup>-1</sup>. The buffer had a final solution concentration of 0.02 M Na<sup>+</sup>, and given the irrigation rates used in this study, we applied 22 kg Na ha<sup>-1</sup> d<sup>-1</sup>. Our previous experience using the tap water (pH  $\sim 9$ , but low in salinity) in the Kansas State greenhouses to establish and maintain STM showed no ill effects on STM growth. Therefore, it seems likely that osmotic stress, induced by sodium, suppressed the growth of STM in these studies, rather than high pH per se. If so, the question is: why was this effect more pronounced at pH's  $\geq 7$ ?

It seems plausible that the sodium tolerance of STM in our studies was enhanced at the lower irrigation water pH's of 5 and 6. Wang et al. (2008) explained that high apoplastic levels

of  $\text{Na}^+$  in bryophytes cause hyperosmotic stress and lead to an ionic imbalance, in which salt-stressed plants must reestablish cellular ion homeostasis via transmembrane proteins, such as sodium ATPase and vacuolar  $\text{H}^+$ -ATPase. Perhaps these vital enzymes were less active, or even denatured, at pH's  $\geq 7$ , but remained intact and active at pH's 5 and 6, enabling the STM to better tolerate the increased salt load.

The availability of most nutrients is highly dependent on soil chemical properties, such as pH; therefore, it is also possible that differences in nutrient availability and uptake are responsible for the large differences in percent cover observed at different irrigation water pH's. Silvery-thread moss is not able to extract nutrients from deep in the soil profile; however, it may absorb solubilized nutrients at the interface between soil and rhizoid. Unfortunately, the mechanisms of nutrient absorption and movement in bryophytes are not well understood (Glime, 2007). If STM is obtaining nutrients from the soil/rhizoid interface, then a nutrient with higher availability in the soil should translate into higher levels in the tissue. This possibility is why we measured nutrient availability in the top 2.5 cm of soil along with the nutrient content in the tissue.

As one might expect, several soil nutrient levels were affected by irrigation water pH, and acid-source again had no effect (data not presented). However, none of the nutrients showing differences in soil availability correlated with tissue nutrient content. In fact, Mg and K were the only tissue nutrients significantly affected by irrigation pH ( $P= 0.003$  and  $<.0001$ , respectively). Magnesium content was actually lower at pH 5 than at higher pH's: STM tissue irrigated with pH's of 7 and 9 contained 0.57% Mg, while STM irrigated with pH 5 water had only 0.38% Mg. Magnesium has many important functions in the plant; therefore, it does not seem likely that it was related to the increased cover we observed at pH's 5 and 6.

Potassium, however, may have played an important role: The amount of K in the tissue increased as pH decreased, with a 2-fold increase in K for pH 5 compared to pH 9 (Figure 5.5). The transport and regulation of potassium is a crucial component for plants undergoing salt stress. During salt stress, several enzymes, such as H<sup>+</sup>-ATPase, actively attempt to maintain a higher K concentration compared to Na<sup>+</sup> in the cytosol (Zhu, 2003). This is necessary because Na and K ions have similar properties, causing Na to compete with K for major binding sites in vital metabolic processes (Shabala and Cuin, 2007). The increased K in gametophyte tissue could potentially explain why mature STM irrigated with sodic pH 5 water did not exhibit any injury, while STM irrigated with pH's 7 and 9 showed visual injury mirroring the concentration of K<sup>+</sup> found in the tissue (Figure 5.6). The association between tissue K content, irrigation water pH, and visual injury (Figures 5.5 and 5.6) makes a strong argument for K<sup>+</sup> playing a role in the increased cover of STM irrigated with sodic pH 5 or 6 water, compared to pH's  $\geq 7$ .

The effect of irrigation pH on STM growth is remarkable considering all solutions supplied pots with 22 kg Na<sup>+</sup> ha<sup>-1</sup> d<sup>-1</sup>. This raises a fundamental question: How did lowering the irrigation pH to 5 or 6 enable STM to better withstand these sodium levels? There are several enzymes involved in plant salt tolerance, and each has a specific pH range at which it operates most efficiently (Michelet and Boutry, 1995). Perhaps reducing the pH of the Na<sup>+</sup> loaded irrigation water increased the activity of these crucial enzymes. Further investigation is required to determine the mechanisms involved that allowed STM to better tolerate this high salt environment when irrigation water pH was reduced. Considering the emphasis currently placed on water conservation and management, it would be worthwhile to investigate whether similar irrigation water pH effects occur with vascular plants; specifically, future research should determine if vascular plants benefit from reducing the pH of sodium-loaded irrigation water.

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Figure 5.1 Shoot tip tissue harvested from pot using curved iris scissors and analyzed for tissue nutrient content.

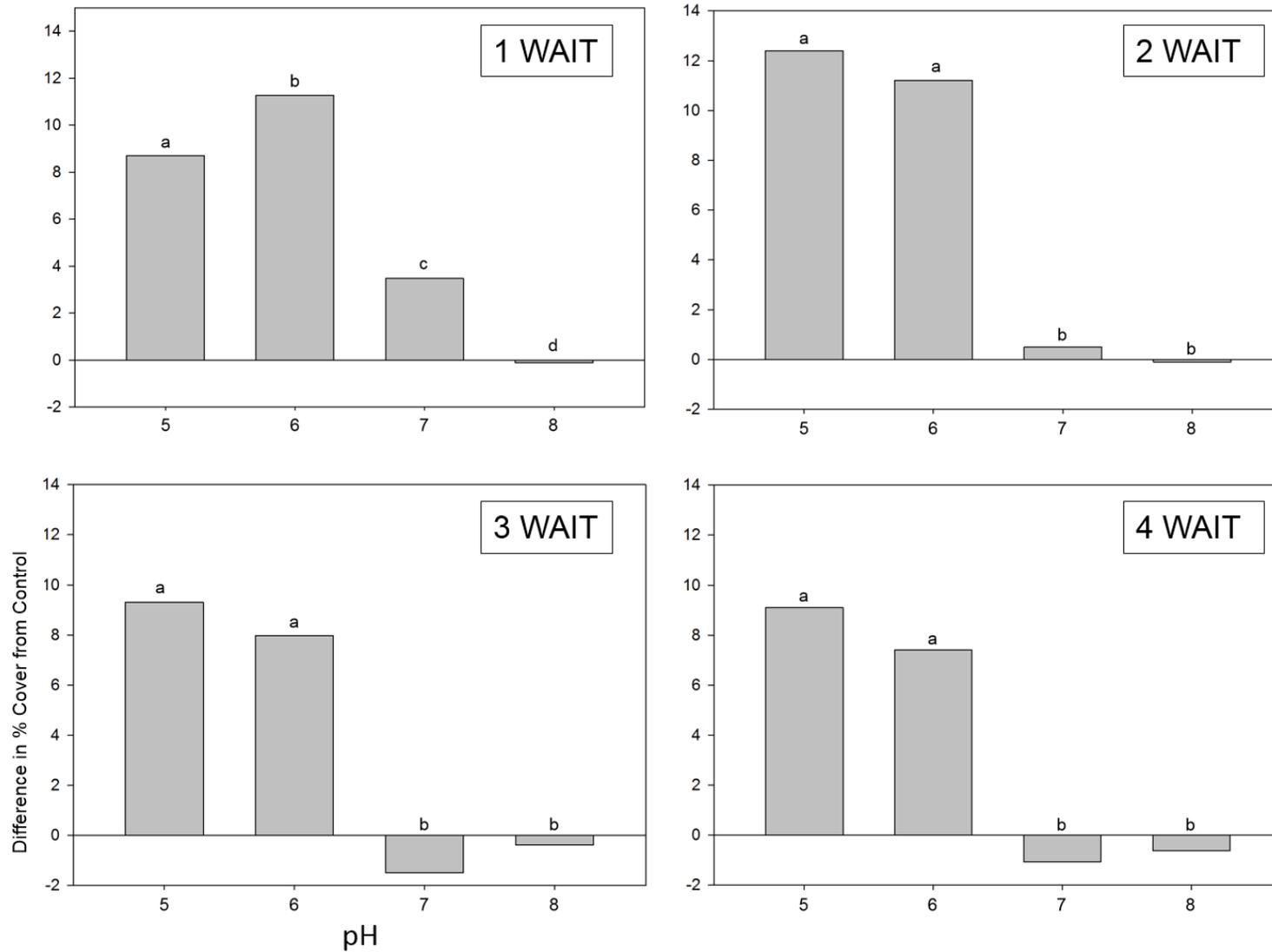


Figure 5.2 Silvery-thread moss (*Bryum argenteum* Hedw.) cover when irrigated with water differing in pH, at one to four weeks-after-initial-treatment (WAIT).

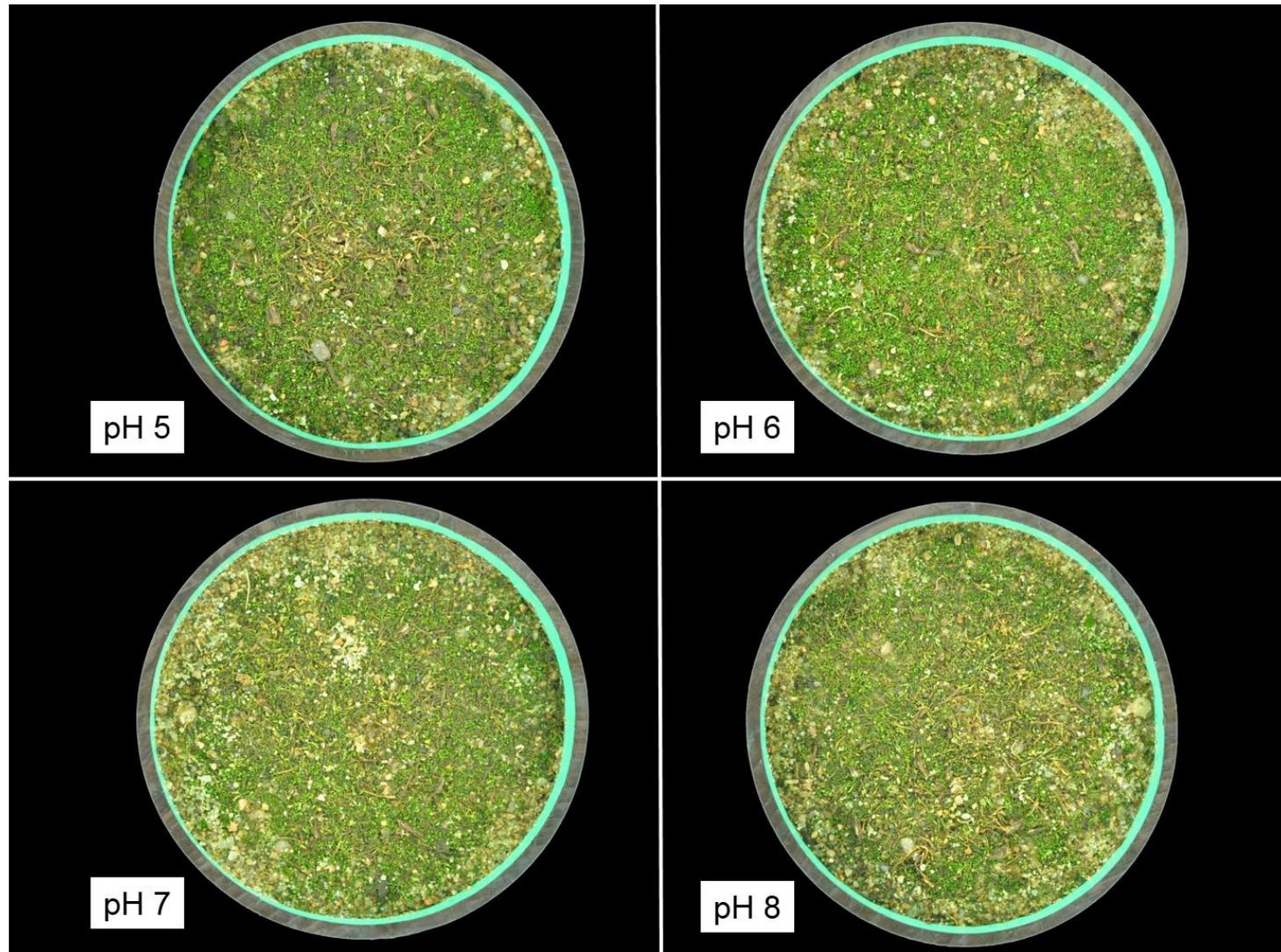


Figure 5.3 Effect of irrigation water pH on silvery-thread moss (*Bryum argenteum* Hedw.) growth at 1 week after initial treatment.

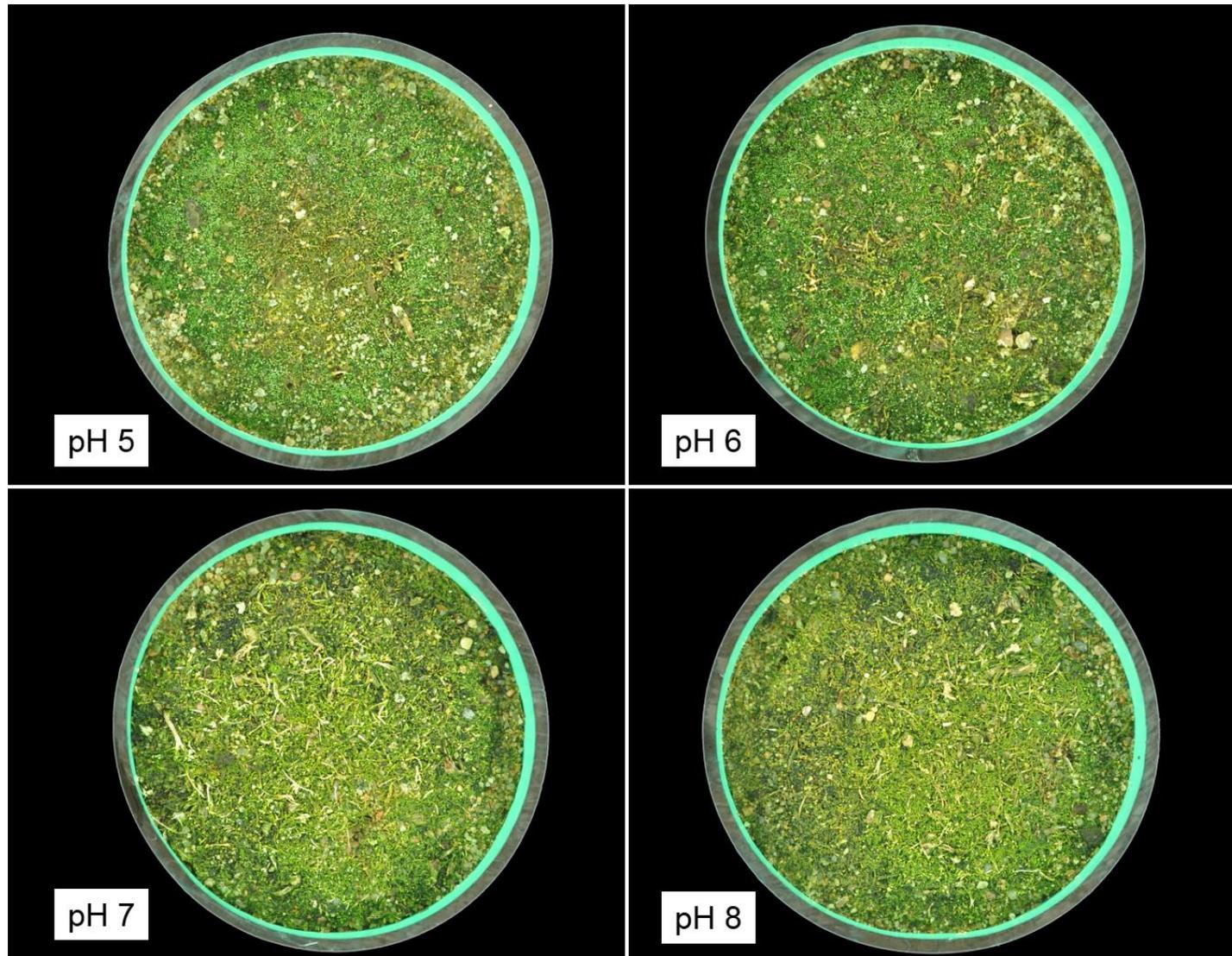


Figure 5.4 Effect of irrigation water pH on silvery-thread moss (*Bryum argenteum* Hedw.) growth at 4 weeks after initial treatment.

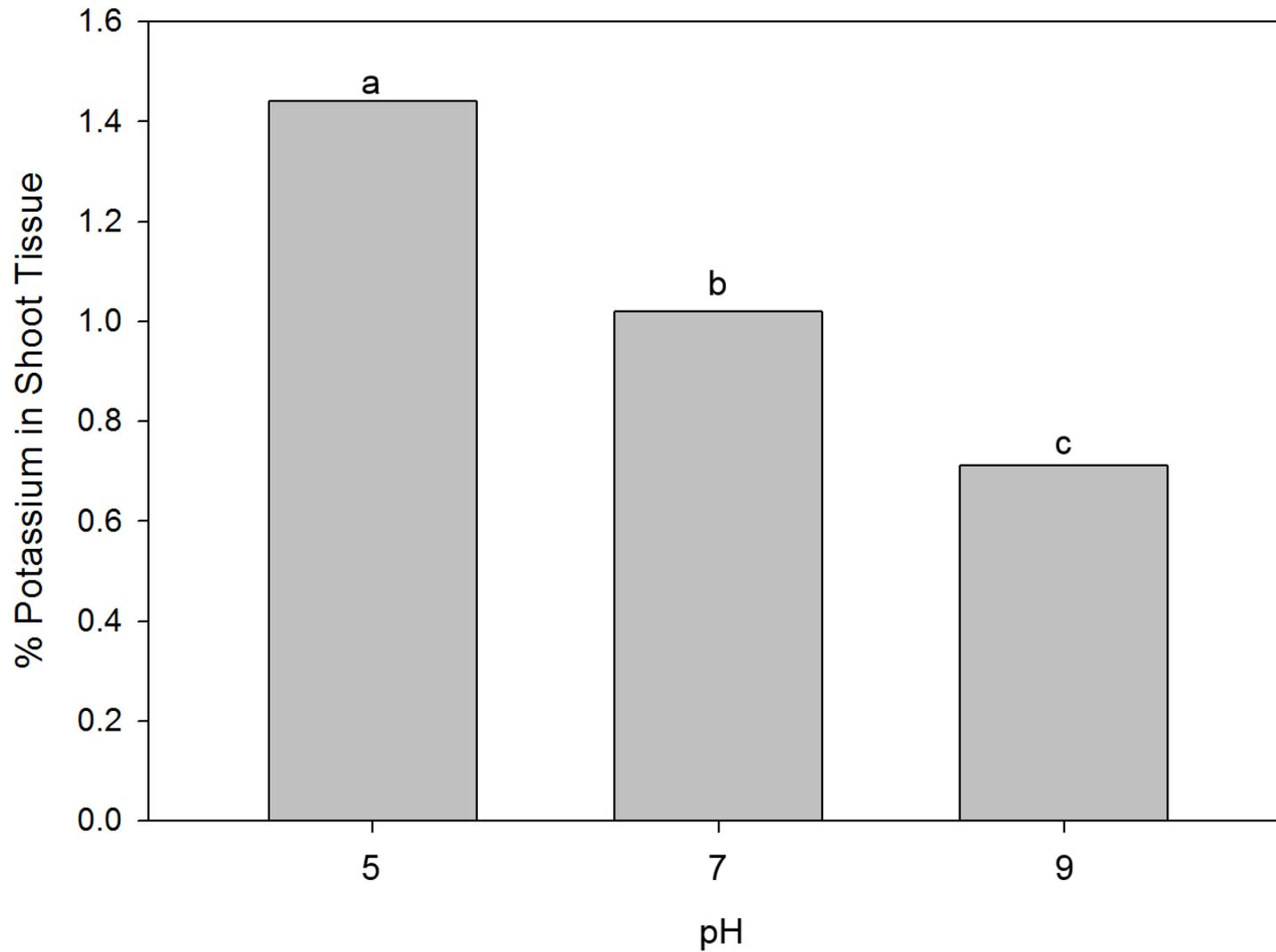


Figure 5.5 Silvery-thread moss gametophyte potassium tissue content as affected by irrigation water pH. Treatments with the same letter above the bar are not significantly different ( $P < 0.05$ ) according to Fisher's protected LSD test.

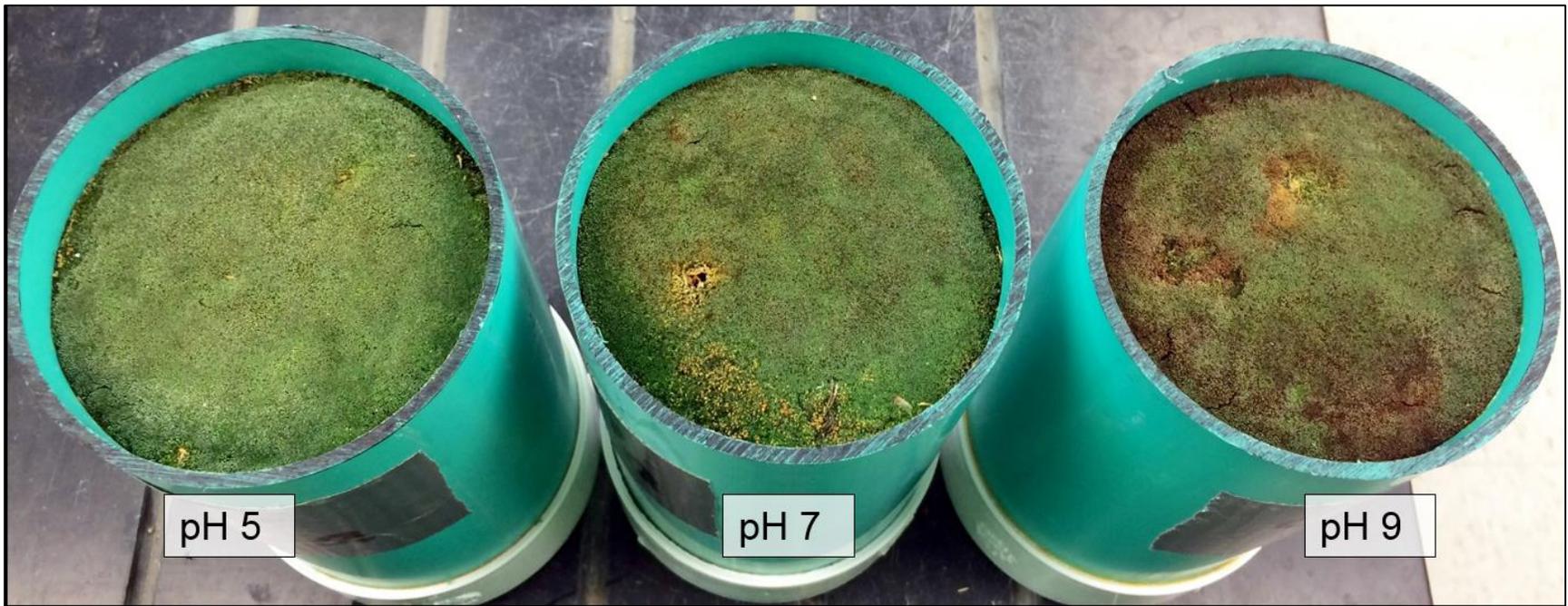


Figure 5.6 Silvery-thread moss visual injury as affected by irrigation water pH after irrigation treatments were applied daily for 14 d.

Table 5.1 Laboratory procedures and extractants used by the Kansas State University Soil Testing Laboratory to determine soil chemical properties and tissue content.

Parameter	Soil Testing Procedure	Tissue Testing Procedure
pH	1:1 (soil:water)	n/a
Phosphorus	Mehlich 3 extraction	Nitric-perchloric acid digest
Potassium	Ammonium acetate extraction	Nitric-perchloric acid digest
Calcium	Ammonium acetate extraction	Nitric-perchloric acid digest
Magnesium	Ammonium acetate extraction	Nitric-perchloric acid digest
Sodium	Ammonium acetate extraction	n/a
Iron	DTPA extraction	Nitric-perchloric acid digest
Manganese	DTPA extraction	Nitric-perchloric acid digest
Zinc	DTPA extraction	Nitric-perchloric acid digest
Ammonium	KCl extraction	KCl extraction
Nitrate	KCl extraction	KCl extraction

Table 5.2 ANOVA for the effects of irrigation water pH and acidifying source at various weeks after initial treatment (WAIT) on silvery-thread moss (*Bryum argenteum* Hedw.) cover.

Source	Num DF	Den DF	Difference in % cover from control	
			F-Value	Pr > F
Acid Source	1	191	0.26	0.6093
pH	3	191	48.54	<.0001
AS × pH	3	191	0.46	0.7127
WAIT	3	191	6.27	0.0004
AS × WAIT	3	191	0.41	0.7490
pH × WAIT	9	191	3.78	0.0002
AS × pH × WAIT	9	191	0.78	0.6341