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Dose responses of silvery-thread moss (*Bryum argenteum*) to carfentrazone-ethyl

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Abstract

Carfentrazone-ethyl is one of few herbicides labeled for control of silvery-thread moss (STM) in golf course putting greens, but common use rates are up to three times higher than for broadleaf weeds. Our objective was to determine the efficacy of a single POST application of carfentrazone-ethyl for STM control in greenhouse and field dose response studies. In the greenhouse, carfentrazone-ethyl was applied at 0, 14, 28, 56, 112, and 224 g ai ha^{-1} to pots containing established STM and creeping bentgrass. Percent gametophyte injury was visually estimated at 14, 28, 49, and 77 d 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_{90} (doses required to cause 90% gametophyte injury) were 26.8 and 54.3 g ha^{-1}, respectively; both of these doses are substantially lower than the label rates for long- and short-term control, respectively. All doses reduced the viability of transplanted shoots at 10 DAT compared to untreated STM; however, regrowth occurred in all Petri dishes by 17 DAT. Field studies were initiated in Manhattan, KS and San Luis Obispo, CA to corroborate greenhouse results. Averaged across locations, carfentrazone-ethyl applied at 56 and 112 g ha^{-1} caused 76% and 84% STM injury at 14 DAT, but STM injury quickly lessened to 45% and 48% by 28 DAT, respectively. In greenhouse and field studies, STM recovery did not occur until 2 wk after treatment (WAT), which indicates the label-stipulated application interval of 2 wk is too short. Our research suggests that 56 g ha^{-1} can provide similar burndown control of STM as compared to the highest label rate (112 g ha^{-1}), and turfgrass managers should consider extending the reapplication interval to 3 or 4 wk when moss recovery is observed.

Introduction

Silvery-thread moss (STM) is a problematic perennial weed in cool-season golf course putting greens, because of its weedy properties and limited control options. It is a cosmopolitan species found on every continent (Crum and Anderson 1981) and a member of the Bryopsida class of bryophytes, which is the most diverse class of mosses (Glime 2007). Silvery-thread moss is capable of not only asexual reproduction via dispersal of dislodged shoot apices, bulbils, and gemmae, but also sexual reproduction via dissemination of microscopic spores (Raudenbush et al 2015). Silvery-thread moss populations growing in putting greens have undergone a divergence in life history traits and exhibit a greater vertical shoot growth rate and 3- to 4-fold increase in shoot density compared to off-green populations; researchers propose that the meticulous management practices used to maintain putting greens may have selected for these traits (Raudenbush et al 2018). The specialized populations of STM growing in putting greens can invade in a single growing season and become increasingly difficult to control as the age and size of the infestation increases (Z. Raudenbush, data not shown).

Carfentrazone-ethyl (Quicksilver®), an aryl triazolinone herbicide, is commonly used for selective control of 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 that, once energized by light, causes the formation of reactive oxygen species (Thompson and Nissen 2000). 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 aryl triazolinone herbicides, use rates required to control weeds are very low (Cobb and Reade 2010).

Carfentrazone-ethyl is a contact herbicide that, once absorbed, can be rapidly hydrolyzed by plants 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, whereas susceptible plants do so to a lesser extent (Dayan et al. 1997). The activity of carfentrazone is rapid, with susceptible plants capable of showing herbicidal symptoms 2 h 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 expedited broadleaf weed control in turfgrass.

Because of STM's unique morphology, only a limited number of herbicidal chemistries are effective against it. Many herbicides require translocation in the xylem and/or phloem to reach their site of action (e.g., synthetic auxins, photosystem II inhibitors, acetolactate synthase inhibitors). Bryophytes lack a true vascular system, as they are unable to synthesize lignin (Glime 2007). Consequently, their simplified conductive tissues most likely prevent several systemic herbicide chemistries from reaching their site of action in lethal quantities. As a contact herbicide, carfentrazone-ethyl does not require translocation through the xylem and/or phloem and is instead absorbed directly through the leaf tissue and into the chloroplast; thus, it most likely has greater efficacy against STM than do other herbicide chemistries.

Quicksilver® has a supplemental label for STM control in creeping bentgrass greens and tees on golf courses. The label stipulates two STM control strategies: (1) for burndown and control of STM in bentgrass greens and tees, apply 112 g ha^{-1}, followed by a second application 2 wk later at the same rate; (2) for control over longer periods, applications may be repeated every 2 wk at a rate not less than 33 g ha^{-1} (Anonymous 2015). Regardless of application strategy, no more than 448 g ha^{-1} can be applied per year. Considering that label application rates for broadleaf weed control are 17 to 35 g ha^{-1}, it is unclear why the application rates for STM control are relatively high (33 to 112 g ha^{-1}). Several researchers have evaluated the efficacy of carfentrazone-ethyl when applied at 112 g ha^{-1} (Borst et al. 2010; Kennelly et al. 2010; Thompson et al. 2011); however, minimal research has evaluated lower use rates. Specifically, Kennelly et al. (2010) applied carfentrazone-ethyl at 56 and 112 g ha^{-1} to creeping bentgrass and reported no differences in STM control between the two rates throughout the study.

The possibility of obtaining STM control with lower carfentrazone-ethyl use rates would be valuable to superintendents from both environmental and fiscal standpoints. Therefore, the objective of this research was to determine the efficacy of a single POST application of carfentrazone-ethyl for STM control at rates ranging from 1/8X to 2X (where X = the approximate label rate of 112 g ha^{-1}).

Materials and Methods

Initial Dose Assessment

Growth chamber studies were conducted from May 2014 to January 2015 to determine the carfentrazone-ethyl dose response in managing STM. The experiment was repeated in time using the same growth chamber. The STM population used in this study was collected from a single colony (<5 cm diam) in a research putting green at Rocky Ford Turfgrass Research Center in Manhattan, KS. The population was vegetatively propagated in the greenhouse for 3 mo to obtain sufficient plant material and was then allowed to air-dry in the laboratory at 19 C for 7 d. After 7 d, dried STM gametophytes (~5 g at one time) were placed in a coffee grinder (Krups F20342, Millville, NJ) and ground for 6 to 7 s to thoroughly shred the plant material.

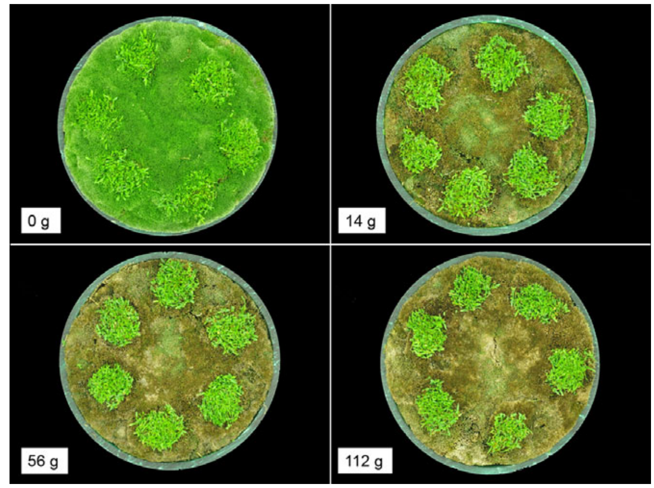

Figure 1. Lightbox images showing silvery-thread moss gametophyte injury at 14 d after treatment from an application of carfentrazone-ethyl. The six small circular green patches are creeping bentgrass plugs. Doses, expressed in g ai ha^{-1}, are shown in bottom-left corners of images. The label rate for silvery-thread moss control in creeping bentgrass putting greens is 112 g ai ha^{-1}.

Polyvinyl chloride containers (10 cm diam 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 US Golf Association recommendations (pH 7.9; CEC 2.75 mEq 100 g^{-1}) 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 h, each pot was planted with 1.2 g of ground STM material and placed in the growth chamber. Plant material was evenly spread over the surface, and then immediately irrigated with 5 mm of water using a misting nozzle (Dramm 610SF, Manitowoc, WI). The misting nozzle was continuously moved while irrigating to prevent puddling of water at the soil surface. Pots received 3 mm of water three times daily for 10 d, and then 5 mm once daily for the remainder of the study. Silvery-thread moss gametophores began actively growing after 7 d. Pots were fertilized weekly throughout the study with a Hoagland 1:10 solution to provide 3 kg N ha^{-1} 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 detached from the sand substrate, curling up at the pot edges. To prevent this from occurring, six 1.3-cm diam by 8-cm creeping bentgrass cv 007 plugs were inserted through the STM gametophyte and into the sand substrate (Figure 1). The plugs and STM were allowed to acclimate for 30 d before any treatments were applied, and plugs were trimmed twice weekly at 4 mm with scissors throughout the duration of the study. Pots were grown in the growth chamber for 90 d before treatments were applied (Conviron E15, Winnipeg, Canada). All pots used in the experiments had 100% cover and an average gametophore length of 2 cm. The growth chamber was set to a day/night temperature of 20 C/17 C and independently measured using temperature loggers (HOBO Pro V2; Onset Computer Corp., Bourne, MA). The chamber was programmed to a 16-h photoperiod and emitted 690 µmol m^{-2} s^{-1} of photosynthetically active radiation according to ceptometer estimates (AccuPar LP-80; Decagon Devices, Pullman, WA) at 30 cm above the pots.

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Carfentrazone-ethyl was applied at rates of 0, 14, 28, 56, 112, and 224 g ha^{-1} using a moving-track spray chamber (Devries Manufacturing, Hollandale, MN) and a XR8002EVS nozzle (TeeJet Technologies, Wheaton, IL) operating at 220 kPa, which delivered a spray volume of 220 L ha^{-1}. All treatments were applied with a nonionic surfactant (AD-SPRAY 90 NIS; Helena Chemical Co., Collierville, TN) at 0.25% v/v. Percent gametophyte injury (0 to 100%) was visually estimated weekly for 11 wk. Plants with necrotic gametophore tips were considered dead, whereas 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 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 trimmed to 1 cm and placed in 51-mm Petri dishes filled with 5 mm sand (as previously described) that had been autoclaved for 20 min at 120 C at a pressure of 98 kPa. 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 d after plating (DAP), the viability of individual shoots was determined by counting the number of erect shoots with more than five 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).

A completely randomized design with four replicates and a one-way treatment structure was used to evaluate the effect of carfentrazone-ethyl dose on measured STM growth parameters for each run. Percent gametophyte injury, gametophyte fresh and dry weight, shoot viability counts, and percent protonema were subjected to ANOVA using the GLM procedure in SAS (SAS, Cary, NC). Experimental run was included in the model, and data were combined when a significant run-by-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 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:

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

where Y is percent gametophyte injury, d is the upper asymptote, b is the slope around e, x is the rate of carfentrazone-ethyl, and e is the rate of carfentrazone-ethyl required to cause 50% of the maximum response. 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).

Field Studies

The lethality of an herbicide typically increases in a controlled environment; therefore, field studies were conducted from June to August in 2015 at the Rocky Ford Turfgrass Research Center in Manhattan, KS, and the Cal Poly Environmental Horticulture Unit in San Luis Obispo, CA, to determine whether growth chamber results could be corroborated. The sites utilized sand-based rootzones containing 'Penncross' and 'L-93' creeping bentgrass, respectively, and were mowed 6 d wk^{-1} at a bench cutting height of 3 mm. Plots were fertilized every 2 wk for the duration of the study with foliar applications of urea (N-P-K; 46-0-0) at a rate of 10 kg N ha^{-1}. Plots were irrigated as needed to prevent drought stress throughout the duration of the experiment.

Both sites had existing STM infestations; however, experimental units were limited. Additionally, pots treated with the 14-g ha^{-1} dose of carfentrazone-ethyl exhibited substantial recovery in the growth chamber, so only 0, 28, 56, 112, and 224 g ha^{-1} doses were evaluated in field studies. Carfentrazone-ethyl doses were applied using a single-nozzle (TeeJet XR8004EVS) CO_2-powered backpack sprayer operating at 207 kPa to deliver a spray volume of 411 L ha^{-1}. A randomized complete-block design with four blocks was used to evaluate the treatments. Blocks were constructed by placing five plots with similar initial percent moss cover in the same block to improve homogeneity of experimental units. Initial STM cover in Manhattan ranged from 4% to 20% with a mean cover of 8.9%, and the STM cover in San Luis Obispo ranged from 4.0% to 15.0% with a mean cover of 9.8%. Percent silvery-thread moss cover of the 0.9-m by 0.9-m plots was determined using a point-intercept method over a 961-intersection grid with 2.54-cm centers. A count was registered if a green, healthy STM gametophyte was positioned directly under an intersection. Relative percent STM control was calculated by comparing counts registered from plots treated with carfentrazone-ethyl to the nontreated plot within each respective block, for example, [(1 − (treated plot count/nontreated plot count)) × 100]. Normality and homogeneity of residual variances were examined using the GPLOT and UNIVARIATE procedures in SAS. Relative percent STM control data were subjected to ANOVA using the MIXED procedure of SAS, and means were separated using Fisher's protected LSD test ($P < 0.05$).

Results and Discussion

Growth Chamber: Initial Dose Assessment

No dose-by-run interaction was present for gametophyte fresh and dry weight, shoot viability, and percent protonema measurements (Tables 1 and 2) in growth chamber studies; therefore, data were combined for those parameters. Additionally, there was no dose-by-run interaction for gametophyte injury data at 28 and 49 DAT, but there was an interaction at 14 and 77 DAT (Table 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 were due to very slight "injury" (<10%) in the nontreated STM from run 2, whereas 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 ha^{-1} (the 1/8X rate) would cause considerably less injury compared to higher doses at 14 DAT, but all doses caused >85% injury (Figure 1); moreover, insights gained from the

Table 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[a], 224 g ai ha^{-1}) of carfentrazone-ethyl.

		P value associated with *F*					
		% Gametophyte injury				Weight	
Source	df	14 DAT	28 DAT	49 DAT	77 DAT	Fresh	Dry
Dose	5	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Run	1	0.0086	0.3651	0.1291	0.001	<0.0001	<0.0001
D × R[b]	5	0.0001	0.7947	0.8386	0.0071	0.3553	0.2336

[a]Highest label rate for silvery-thread moss control in creeping bentgrass putting greens.
[b]Abbreviations: D, dose; R, run.

Table 2. ANOVA for total number of erect shoots with more than five leaves, and percent of shoot covered with protonema when harvested from STM treated with differing doses (0, 14, 28, 56, 112[a], 224 g ai ha^{-1}) of carfentrazone-ethyl at 7 and 14 d after treatment (DAT).

		P value associated with *F*			
		Total shoots		% Protonema	
Source	df	7 DAT	14 DAT	7 DAT	14 DAT
Dose	5	<0.0001	0.0340	0.0464	0.5111
Run	1	0.1519	0.0207	0.0034	0.2520
D × R[b]	5	0.4863	0.7088	0.5816	0.8440

[a]Highest label rate for silvery-thread moss control in creeping bentgrass putting greens.
[b]Abbreviations: D, dose; R, run.

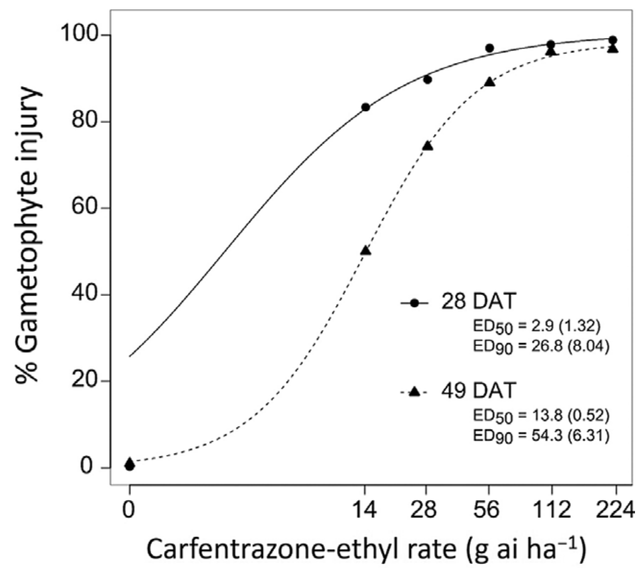

Figure 2. Percent silvery-thread moss gametophyte injury, and ED$_{50}$ and ED$_{90}$ values, as influenced by carfentrazone-ethyl application rate at 28 and 49 d after treatment (DAT). Values within parentheses are the standard error (±) for each ED value as predicted by the log-logistic model. The label rate for silvery-thread moss control in creeping bentgrass putting greens is 112 g ai ha^{-1}.

77-DAT data were not substantially different from those obtained from the 49-DAT data. The main difference was that overall injury levels at 77 DAT were lower for all doses, as STM continued to recover.

The Quicksilver label for STM control stipulates herbicide reapplication 14 d after the initial treatment using a rate between 33 and 112 g ha^{-1}. In the growth chamber, significant STM injury was observed at 14 DAT for all doses, and recovery did not occur until >21 DAT (Figure 1). At 28 DAT, the estimated ED$_{90}$ value for gametophyte injury was 26.8 g ha^{-1} (Figure 2). At 49 DAT additional recovery had occurred, and the 14 g ha^{-1} rate had only 50% gametophyte injury (Figures 2 and 3) and the ED$_{90}$ value was 54.3 g ha^{-1}, which was a 2-fold increase compared to the ED$_{90}$ value at 28 DAT. These preliminary dose responses suggest that the ideal carfentrazone-ethyl application rate may be dependent on the reapplication interval; thus, superintendents who apply every 14 d may utilize the lowest labeled rate of 33 g ha^{-1}, and those who apply every 28 d utilize a higher application rate.

Silvery-thread moss gametophyte biomass was also affected by carfentrazone-ethyl dose in growth chamber studies (Table 1). Nontreated STM had higher fresh- and dry-biomass accumulation compared to those treated with carfentrazone-ethyl (Figure 4). There were no differences in biomass accumulation of STM among carfentrazone-ethyl doses tested (Figure 4); although several doses caused >90% visible gametophyte injury, that injury was not accompanied by proportionate decreases in fresh and dry weight. This result demonstrates an ability of STM gametophytes to resist decay after being injured by carfentrazone-ethyl. After being treated with herbicides, most dead vascular plants eventually lose their turgidity, collapse, and begin to decompose. However, bryophytes decompose more slowly than do 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-ethyl alone for STM control may be unsuccessful: Bentgrass stolons and tillers may have difficulty penetrating the dense STM gametophyte, even after STM has been injured by carfentrazone-ethyl. Therefore, following carfentrazone-ethyl 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 2017). 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 vegetatively through dislodged shoots, increasing the size of an infestation (Raudenbush et al. 2015). If the viability of dislodged shoots could be reduced by carfentrazone-ethyl, then superintendents could make an application before implementing practices that are likely to dislodge shoots (e.g., aerification, verticutting, grooming, brushing), to reduce the spread and establishment of new colonies. In growth chamber studies, carfentrazone-ethyl did affect the viability of individual shoots (Table 2 and Figure 5). At 7 DAP (corresponding to 10 DAT), Petri dishes containing shoots harvested from nontreated STM had a mean count of 7.8 shoots with more than five

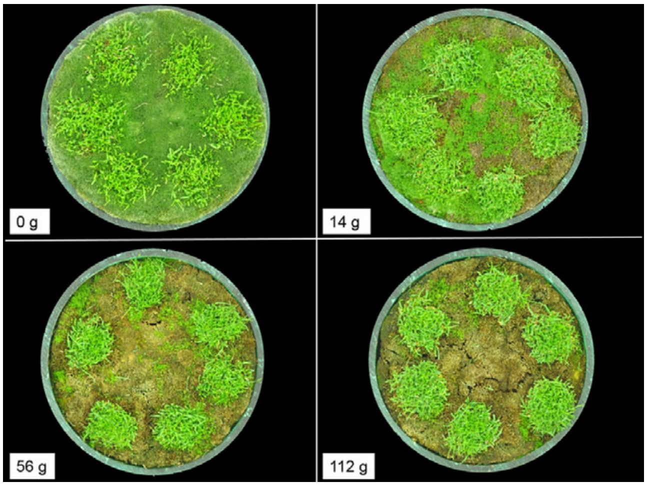

Figure 3. Lightbox images showing silvery-thread moss gametophyte injury at 49 d after treatment from an application of carfentrazone-ethyl. Doses, expressed in g ai ha^{-1}, are shown in bottom-left corners of images. The label rate for silvery-thread moss control in creeping bentgrass putting greens is 112 g ai ha^{-1}.

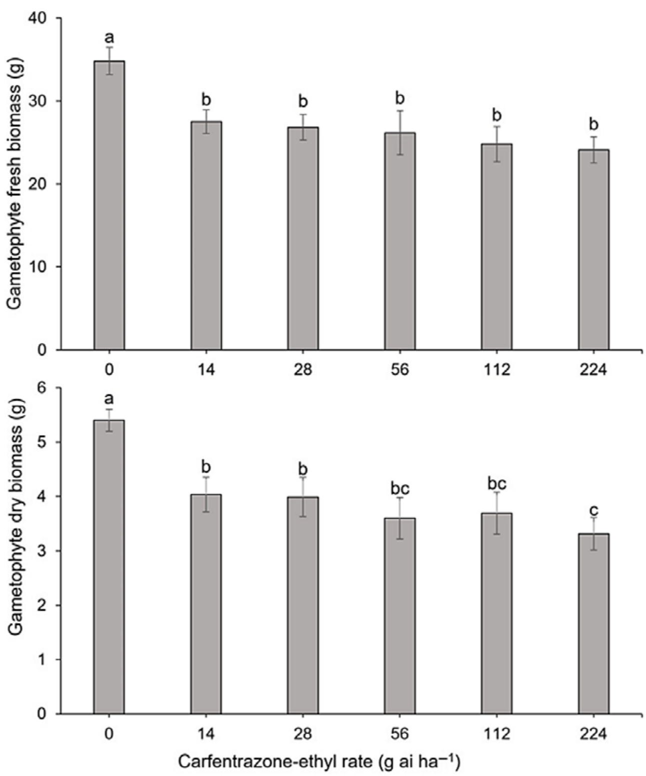

Figure 4. Effect of carfentrazone-ethyl on silvery-thread moss gametophyte fresh and dry biomass harvested at 77 d after treatment. Within each graph, treatments with the same letter above the bar are not significantly different ($P < 0.05$) according to Fisher's protected LSD test.

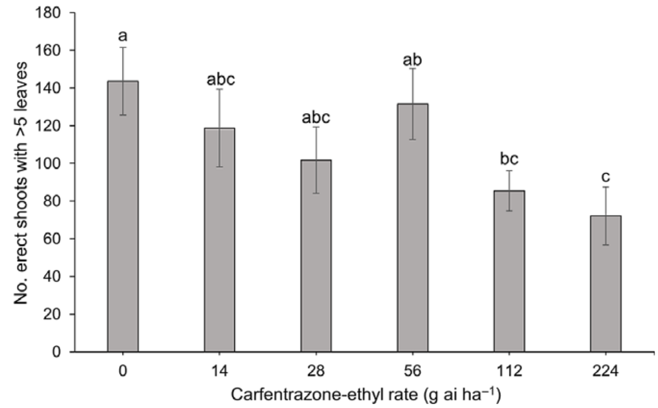

Figure 5. Viability of eight plated silvery-thread moss shoots 17 d after being harvested from pots treated with differing rates of carfentrazone-ethyl. Treatments with the same letter above the bar are not significantly different ($P < 0.05$) according to Fisher's protected LSD test.

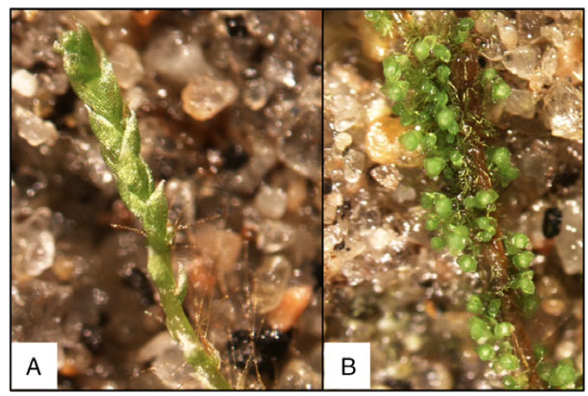

Figure 6. Silvery-thread moss (STM) shoots excised from carfentrazone-ethyl–treated STM in a growth chamber. A, Untreated shoot producing rhizoids and exhibiting positive phototropism; B, shoot excised from STM treated with carfentrazone-ethyl at 56 g ai ha^{-1} producing protonema and new leaf primordia.

leaves, whereas shoots harvested from STM receiving any carfentrazone-ethyl dose had none (data not shown). The erect shoots in nontreated pots at 7 DAP were not new leaf primordia; rather, the existing gametophore tip produced rhizoids and exhibited positive phototropism (Figure 6). Furthermore, 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. In addition, although no carfentrazone-ethyl–treated STM had erect shoots with more than five leaves at 7 DAP, nearly all of the excised shoots (from all carfentrazone-ethyl doses) were producing new leaf primordia and protonema (Figure 6B).

By 14 DAP, all Petri dishes contained a large number of erect shoots; however, the 112 and 224 g ha^{-1} rates of carfentrazone-ethyl reduced the number of shoots compared to the nontreated (Figure 5). This raises an interesting question: Why did the shoots in Petri dishes exhibit such prolific and rapid regrowth at 18 DAT, but the intact gametophytes took ≥28 d to exhibit recovery in the growth chamber? Two scenarios seem plausible: First, although it was not directly measured, relative humidity in the covered Petri dishes is likely near 100%, whereas 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 5), which reinforces the importance of

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Table 3. ANOVA for relative percent silvery-thread moss control at 2, 4, and 6 wk after treatment (WAT) when sprayed with differing doses (28, 56, 112[a], 224 g ai ha^{-1}) of carfentrazone-ethyl in creeping bentgrass putting greens in Manhattan, KS and San Luis Obispo, CA.

		P value associated with *F*		
		Relative % moss control		
Source	df	2 WAT	4 WAT	6 WAT
Dose	3	0.0001	0.0059	0.0812
Location	1	0.0106	0.0381	0.1339
D × L[b]	3	0.8865	0.2298	0.9626

[a]Highest label rate for silvery-thread moss control in creeping bentgrass putting greens.
[b]Abbreviations: D, dose; L, location.

Table 4. Main effect of carfentrazone-ethyl dose (28, 56, 112[a], 224 g ai ha^{-1}) on relative percent silvery-thread moss control at 2, 4, and 6 wk after treatment (WAT) averaged across locations in Manhattan, KS and San Luis Obispo, CA.

	Relative % moss control		
Dose	2 WAT[b]	4 WAT	6 WAT
224	95 a	55 a	59
112	84 ab	48 a	57
56	76 b	45 a	46
28	52 c	23 b	25

[a]Highest label rate for silvery-thread moss control in creeping bentgrass putting greens.
[b]Doses with the same letter within a column are not significantly different ($P < 0.05$) according to Fisher's protected LSD test.

taking action in the early stages of an STM infestation, as a colony 5 cm in diameter can contain thousands of individual shoots.

Dose Responses in the Field

In field studies, a dose-by-location interaction was not present at any rating date, but the main effects of dose and location were both significant at 2 and 4 WAT; treatments were not different at 6 WAT at either location (Table 3). The location effect was due to greater STM control in California compared to Kansas at 2 and 4 WAT (data not shown). For instance, at 2 WAT the average STM control in California ranged from 63% to 100% (lowest to highest dose), whereas in Kansas control ranged from 40% to 89%. At 4 WAT, silvery-thread moss was recovering, and control ranged from 33% to 70% in California and from 14% to 46% in Kansas. Overall, the dose response of STM to carfentrazone-ethyl was similar across locations (e.g., increasing the dose caused greater STM control); therefore, STM control data were combined across locations (Table 4).

Averaged across locations, 56 and 112 g ha^{-1} caused 76% and 84% STM control at 2 WAT, respectively; this was greater than the 52% injury caused by 28 g ha^{-1} (Table 4). By 4 WAT, STM recovery was observed, and control was reduced to 45% and 48% when treated at 56 and 112 g ha^{-1} doses, respectively. Applying 222 g ha^{-1} caused greater injury at 2 WAT compared to 56 g ha^{-1}, but no differences were observed between the treatments thereafter. At 6 WAT, significant STM recovery had occurred and no differences in control were observed for any dose. No differences in STM control were observed between the 56 and 112 g ha^{-1} doses throughout the duration of the field studies. The ability of STM to resist decay should be considered when interpreting results from the field studies. For instance, when collecting percent control data, a count was registered if a green, healthy STM gametophyte was positioned under an intersect of the rating grid; an injured STM gametophyte did not register a count. At 2 WAT, 56 and 112 g ha^{-1} doses of carfentrazone-ethyl injured a majority of the moss gametophytes, so that relative percent control was high; however, STM's ability to resist decay allowed the injured gametophytes to persist within the turfgrass canopy, and regrowth occurred directly from the injured gametophytes, which were subsequently counted at the 4- and 6-WAT rating dates. By 6 WAT, a majority of the STM had recovered, and relative percent control ranged from 25% to 59%. Ultimately, an application of carfentrazone-ethyl is highly effective at injuring STM, but the effects are temporary, and regrowth can occur from gametophytes that remain in the turfgrass canopy.

The ED_{50} and ED_{90} values estimated from greenhouse studies and results from field experiments suggest that lower doses of carfentrazone are effective at injuring STM (Figure 2 and Table 4). Additionally, the current Quicksilver label stipulates spraying carfentrazone-ethyl at 2-wk intervals. In our research, no regrowth was observed until after 2 WAT. Superintendents should closely monitor gametophyte colonies following an application of carfentrazone-ethyl. This herbicide inhibits an enzyme involved in chlorophyll production; therefore, if STM has no green, healthy tissue, then minimal target sites will be available for further injury. Superintendents should consider extending the application interval to 3 or 4 wk, by which time some regrowth will likely have occurred.

The ability of carfentrazone-ethyl 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-ethyl about 1 wk before the implementation of canopy-invasive practices, such as core-aerification, verticutting, and brushing. Because the carfentrazone-ethyl–treated shoots in our study showed the ability to regenerate, a follow-up application 2 wk after canopy-invasive practices will help control any dispersed plant material.

Finally, carfentrazone-ethyl is an extremely valuable tool for reducing the invasive 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. Our research supports incorporating the strategic use of carfentrazone-ethyl when attempting to manage a STM infestation; however, superintendents who rely solely on applications of carfentrazone-ethyl to eliminate this pest may find their efforts to be unsuccessful.

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