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Preemergence Control of Silvery Threadmoss (*Bryum argenteum*) Grown from Spores and Bulbils in Axenic Culture

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Silvery threadmoss naturally reproduces through spore and bulbil production, both of which have potential to be controlled prior to establishment. Studies have not evaluated effects of turf protection products on moss protonema or gametophyte growth from spores or bulbils; consequently, most moss is controlled POST on putting greens. Initial studies were performed to determine the optimal growth temperature for spores and bulbils in sterile culture. Protonemata from spores grew optimally at 29.5 C and gametophytes from bulbils grew optimally at 22.5 C. Three subsequent in vitro studies were conducted to evaluate effects of turf protection products on moss development from spores or bulbils in axenic culture at a constant 24 C. Carfentrazone, which effectively controls mature silvery threadmoss gametophytes POST, also reduced green cover of moss protonemata and gametophyte production from spores and bulbils. All combinations with carfentrazone reduced area under the progress curve (AUPC) for green cover of moss for both spores and bulbils by 80% or more by 3 wk after treatment. Sulfentrazone, oxyfluorfen, oxadiazon, saflufenacil, flumioxazin, and pyraflufen-ethyl reduced AUPC of moss equivalent to carfentrazone for both propagule types. The two fosetyl-Al products, phosphite, and mineral oil caused an increase in silvery threadmoss cover between 22 and 113% of the nontreated for spores; however, only methiozolin positively influenced AUPC (90.2%) compared to the nontreated for bulbils. Though silvery threadmoss is typically targeted POST on putting greens, there are products that can provide PRE control, including the industry standard of carfentrazone. These data suggest that differences may occur between turf protection products in their ability to suppress silvery threadmoss establishment from spores or bulbils.
Nomenclature: Carfentrazone; flumioxazin; fosetyl-Al; methiozolin, 5-(2,6-diflourobenzyloxymethyl)-5-methyl-3-(3-methylthiophen-2-yl)-4,5-dihydro-isoxazole; mineral oil; oxadiazon; oxyfluorfen; phosphite; pyraflufen-ethyl; saflufenacil, 2-Chloro-5-[3,6-dihydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)-1(2H)-pyrimidinyl]-4-fluoro-N-[[methyl(1-methylethyl)amino]sulfonyl]benzamide; sulfentrazone; silvery threadmoss; *Bryum argenteum* Hedw.
Key words: Moss control, putting green, silver moss.

Bryum argenteum es un musgo que se reproduce naturalmente mediante esporas y pequeños bulbos, y para ambos hay potencial de control antes del establecimiento. No hay estudios que hayan evaluado los efectos de productos de protección para céspedes sobre el crecimiento del musgo en estados de protonema y gametofítico a partir de esporas o bulbos. Consecuentemente, la mayoría de los musgos son controlados POST en putting greens. Estudios iniciales fueron realizados para determinar la temperatura para el crecimiento óptimo de esporas y bulbos en un medio estéril. Se creció protonema a partir de esporas óptimamente a 29.5 C y gametofitos a partir de bulbos a 22.5 C. Luego se realizaron tres estudios in vitro para evaluar los efectos de productos de protección para céspedes sobre el desarrollo del musgo en un cultivo axénico a una temperatura constante de 24 C. Carfentrazone, el cual controla efectivamente gametofitos maduros de *B. argenteum* en aplicaciones POST, también redujo la cobertura verde de protonometa del musgo y la producción de gametofitos a partir de esporas y bulbos. Todas las combinaciones con carfentrazone redujeron el área bajo la curva de progreso (AUPC) de la cobertura verde del musgo tanto para esporas como para bulbos en 80% o más a 3 semanas después del tratamiento. Sulfentrazone, oxyfluorfen, oxadiazon, saflufenacil, flumioxazin, y pyraflufen-ethyl redujeron AUPC del musgo a niveles equivalentes a carfentrazone para ambos tipos de propágulo. Los dos productos de fosetyl-Al, phosphite, y aceite mineral causaron un incremento en la cobertura del musgo entre 22 y 113% con comparación al testigo sin tratamiento para esporas. Sin embargo, solamente methiozolin influenció positivamente AUPC (90.2%) al compararlo con el testigo sin tratamiento en el caso de los bulbos. Aunque *B. argenteum* es típicamente controlado POST en putting greens, hay productos que pueden brindar control PRE, incluyendo el estándar de la industria, carfentrazone. Estos datos sugieren que diferencias pueden ocurrir entre productos de protección para césped con respecto a su habilidad para suprimir el establecimiento del musgo *B. argenteum* a partir de esporas o bulbos.

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Silvery threadmoss is a problematic weed of golf course putting green turf (Burnell et al. 2004; Cook et al. 2002; Happ 1998) capable of producing three propagule types including spores, bulbils, and

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fragments with varying longevity (Best 1904; Miles and Longton 1992). Frequent mowing allows propagules to spread easily across the surface of the green and from one green to another. Constant irrigation and very low heights of cut make putting greens a prime environment for silvery threadmoss colonization. Silvery threadmoss is typically managed POST and there are no herbicides labeled for PRE control.

During sexual reproduction spores are produced by the sporophyte (Figure 1). Sporulation rarely occurs on putting greens since the spore capsule is borne on a stalk that is approximately 2 cm long and mowing would remove it before maturity (Crum and Anderson 1981). In Virginia, and throughout much of the Southeast, most putting greens are mowed between 3 and 4 mm so it is unlikely that sporulation could occur (GCSAA 2012). However, spores may be produced by colonies nearby a putting green in higher heights of cut. Spores are spread by wind and water so they may come from long distances to infest a putting green (Miles and Longton 1992). Silvery threadmoss is a ubiquitous species with small spores, < 10 μm in diameter, which may be found in the higher atmosphere in global air currents (Frahm 2007). These can settle out at any time depending on atmospheric conditions. In this way spores may come from miles away. The most common way silvery threadmoss spreads around a putting green is through the two types of vegetative propagules, fragments and bulbils (Best 1904; Figure 1). Silvery threadmoss fragments are created by mowers and other equipment which are used daily on most putting greens. Each healthy silvery threadmoss fragment, as small as only a few cells, can regenerate an entire plant (Best 1904; Smith 1999). Bulbils are a vegetative reproductive structure formed by silvery threadmoss; they consist of a few leaves attached to a central stem with no rhizoid. It is unclear what physiological or environmental conditions trigger the development of bulbils, but in research plots at Virginia Tech (Blacksburg, VA), they have been observed to form on silvery threadmoss in early spring and after periods of stress such as aerification, topdressing, and verticutting. The bulbils are easily released mechanically by splashing water, movement of equipment, or foot traffic and they can presumably be moved from putting green to putting green by each of these means. They float and can move in water as well (Rudolph 1970; Smith 1999).

Though many other weeds, especially those with small propagules, are commonly controlled with PRE herbicides, no products are labeled for PRE silvery threadmoss control and superintendents have only limited chemical options for POST management. PRE control options would be valuable to the golf industry. The physiology of bryophytes is different from that of vascular plants and many products used for vascular plant control may not affect silvery threadmoss growth. Some studies have evaluated pesticides or fertilizers for moss control but most have focused on POST control. For example, Burnell et al. (2004) evaluated combinations of iron- and nitrogen-containing fertilizers, oxadiazon, and chlorothalonil (2,4,5,6-tetrachlorobenzene-1,3-dicarbonitrile) with and without zinc. Chlorothalonil is labeled and commonly used as a turfgrass fungicide; however, in these evaluations it functions as a herbicide. These evaluations indicated silvery threadmoss populations could be reduced with chlorothalonil or iron-containing fertilizers as much as 87% 6 wk after treatment. Fausey (2003) tested a wider range of products in soilless media and identified several potential treatments including oxyfluorfen, flumioxazin, and pelargonic acid; however, several of these treatments would be injurious to creeping bentgrass (*Agrostis stolonifera* L.) putting green turf. More recent work by Borst et al. (2010) evaluated mancozeb plus copper hydroxide and carfentrazone applied with and without topdressing and nitrogen fertilizers. They found carfentrazone at 0.12 kg ha^{-1} followed by nitrogen and topdressing to be the most effective treatment, controlling silvery threadmoss 78% up to 16 wk after treatment. Additional POST work has been conducted looking at similar treatment combinations. Boesch and Mitkowski (2005) also found carfentrazone to be 90% effective applied sequentially at a 14-d interval. However, Cook et al. (2002) and Thompson et al. (2011) investigated alternative control products and found copper hydroxide and sodium bicarbonate effective for moss control, respectively. However, a broader evaluation of herbicides, fungicides, plant growth regulators, and fertilizers on silvery threadmoss growth and development is needed particularly for identifying effective PRE treatments.

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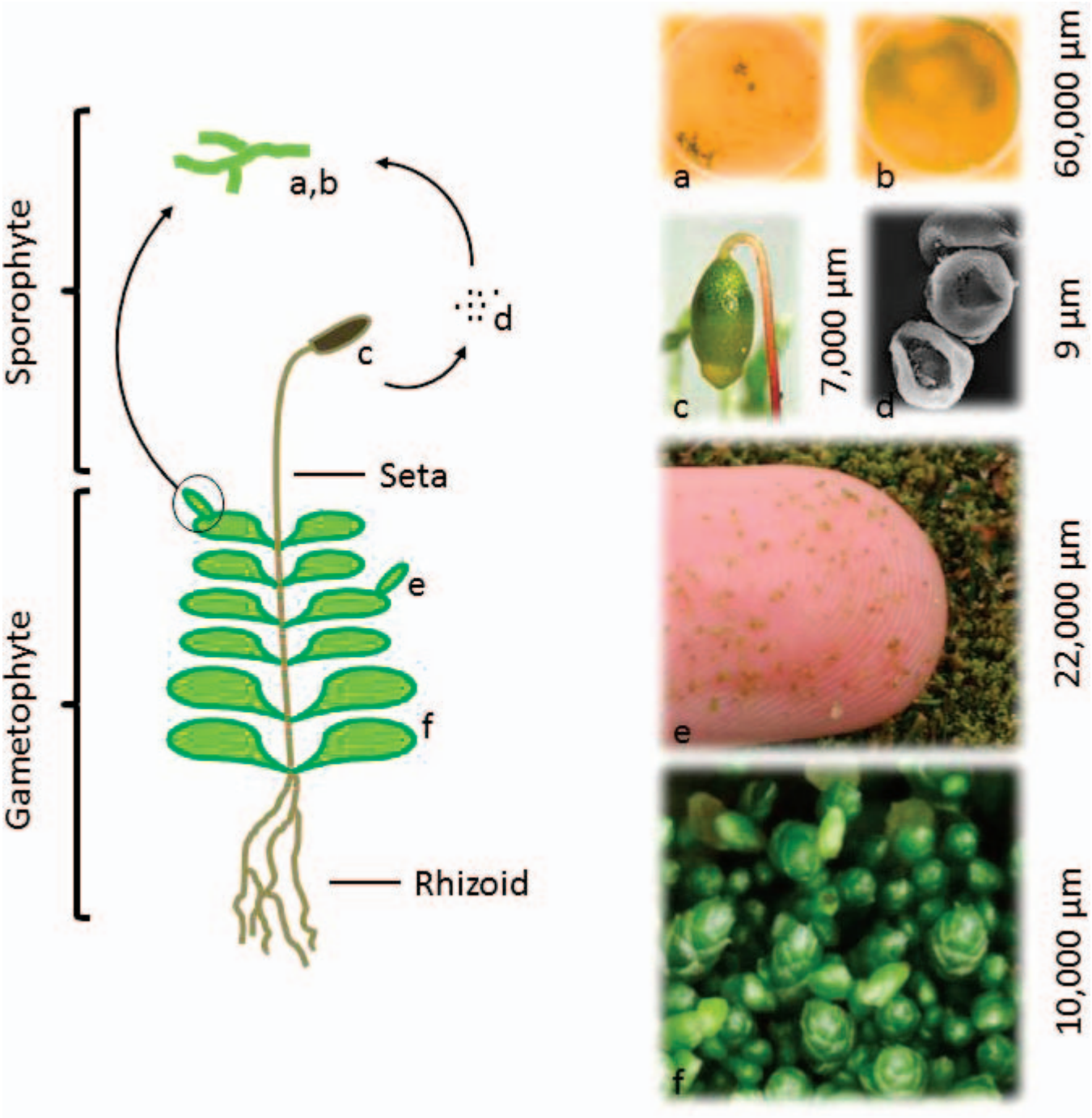

Figure 1. Representation of silvery threadmoss life cycle. (a) Protonemata formed from bulbils 14 d after plating into Phytagel media; (b) protonemata formed from spores 14 d after plating into Phytagel media; (c) spore capsule formed on mature sporophyte growth phase; (d) scanning electron micrograph of silvery threadmoss spores; (e) detached bulbils collected from a creeping bentgrass putting green in Blacksburg, VA; (f) leafy gametophyte tissue with fully formed bulbils ready for vegetative propagation. Each pictured structure is identified by its respective letter in the illustration.

The first step in screening large numbers of products for PRE control is to develop simple methods for culture of silvery threadmoss in growth chamber, greenhouse, and laboratory settings. Establishing axenic cultures from surface-sterilized spore capsules is the most commonly used method to obtain sterile bryophyte cultures, and methods have been described for many species,

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mostly for the purposes of conservation and restoration (Basile and Basile 1988; Duckett et al. 2004; Rowntree 2006; Sabovljevic et al. 2010; Sargent 1988). Murashige and Skoog (MS) medium (Murashige and Skoog 1962) is the most common medium used and it can be adjusted with many additives to meet the specific needs of each species. Though Sabovljevic et al. (2002) presented axenic methods for culturing silvery threadmoss from spore capsules and gametophyte shoots, it was a short communication and did not fully describe comprehensive methods for culturing silvery threadmoss for the intentions of research. Jones and Rosentreter (2006) described methods for growing three moss species including silvery threadmoss on artificial substrates and native soils for the purposes of restoration. But these methods lacked important details such as optimum temperature for growth. Liang et al. (2010) also described methods for culturing silvery threadmoss in liquid media in order to produce moss gametophores for commercial applications; however, liquid suspensions of silvery threadmoss would not accurately simulate field conditions where this species reproduces on a solid soil substrate.

Therefore, a main objective of this work was to determine the optimal temperature for sterile culture of silvery threadmoss from spores and bulbils. This method would facilitate research with the primary objective of evaluating herbicide, fungicide, and combination treatments for PRE control of bulbils and spores, both of which are propagules naturally formed by silvery threadmoss. Our expectation was that silvery threadmoss growth would increase with increasing temperatures and plant protection products commonly used on putting greens would vary in their ability to prevent establishment of silvery threadmoss colonies from bulbils or spores.

Materials and Methods

Bulbil Preparation for Axenic Culture. Silvery threadmoss bulbils (Figure 1) were collected in March and December 2011 and placed in cold storage at 4 C until trials were initiated in summer 2012. In a laminar flow hood, bulbils were surface-sterilized with 10% bleach for 2 min and then rinsed twice in sterile deionized water for 2 min each time. Local tap water was deionized with a Barnstead deionizer (Capital Scientific, Austin, TX) and sterilized at 240 C for 20 min. Bulbils were then rinsed into a 100-mm polystyrene petri dish (Fisher Scientific, Pittsburgh, PA) of sterile water where they retained the ability to float even after sterilization. Approximately 50 bulbils were removed from the surface of the water and rinsed onto 60-mm petri plates with a pipette stream of 400 µl sterile water. Plates were swirled on the benchtop to evenly distribute the water and bulbils over the surface. Each 60-mm plate contained a 4-mm depth of MS medium plus Gamborg vitamins (MP Biomedicals LLC, Solon, OH) in a 0.7% Phytagel (Sigma Chemical Co., St. Louis, MO). Plates were sealed with Parafilm (The Lab Depot, Dawsonville, GA) to prevent moisture loss.

Spore Preparation for Axenic Culture. Silvery threadmoss spore capsules were collected in March 2011 and March 2012 and placed in cold storage at 4 C until experiments were initiated in summer 2012. In a laminar flow hood sporophytes were surface-sterilized in a 10% bleach solution for 2 min. Capsules were then rinsed twice in 1 ml of sterile water for 1 min each time. Twenty sterile capsules were placed in 5 ml sterile water and crushed with a sterile pipette tip. Approximately 3,000 spores were present in 100 µl of the resulting suspension, estimated by counting 10 µl under the light microscope. One hundred microliters of the spore suspension was plated into the center of 100-mm petri plates and 600 µl of sterile water was added to allow for more even spreading of spores on the plate. Plates were swirled to spread the suspension evenly on the plate. MS medium and Gamborg vitamins (MP Biomedicals LLC) were used with the following preliminary medium amendments: none, + 1.5% sucrose, + 1.5% mannose, + 1.5% glucose, and + 1.5% mannitol. Only the nonamended medium stimulated spore germination. Subsequent studies were performed with MS medium and Gamborg vitamins only and all media were solidified using 0.7% Phytagel (Sigma Chemical Co.).

Temperature Response. Experiments for bulbils were separate from those for spores. Experiments were arranged as a split plot with three replications. Each experiment was repeated twice for a total of

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Table 1. Sources of chemicals used in PRE testing of bulbils versus spores. For rates and product mixtures evaluated please see Table 2.

Common name	Trade name[a]	Company	City, state	Web site
Carfentrazone	Quicksilver	FMC Corp.	Philadelphia, PA	http://www.fmccrop.com
Chlorothalonil zinc	Daconil Zn	Syngenta	Greensboro, NC	http://www.syngenta-us.com
Flumioxazin	Sureguard	Valent USA Corp.	Walnut Creek, CA	http://www.valent.com
Fosamine-ammonium	Krenite	Hubei Sanonda Co.	Jingzhou Hubei, China	http://www.sanonda.cn
Fosetyl-Al (QP)	Fosetyl-Al	Quali-Pro	Raleigh, NC	http://www.quali-pro.com
Fosetyl-Al (Sig.)	Chipco Signature	Bayer CropSciences	Research Triangle Park, NC	http://www.bayercropscienceus.com
Mancozeb + copper hydroxide	Junction	SePRO Corp.	Carmel, IN	http://www.sepro.com
methiozolin	PoaCure	Moghu Research Center	Daejeon, South Korea	http://www.moghu.com
Mineral oil	Equate mineral oil	PL Developments	Westbury, NY	http://www.pldevelopments.com
Oxadiazon	Ronstar 2G	Bayer CropScience	Research Triangle Park, NC	http://www.bayercropscienceus.com
Oxyfluorfen	Goal	Dow AgroSciences,	Indianapolis, IN	http://www.dowagro.com
Phosphite	Fosphite	JH Biotech Inc.	Ventura, CA	http://www.jhbiotech.com
Propaconizole	Banner MAXX	Syngenta	Greensboro, NC	http://www.syngenta-us.com
Pyraflufen-ethyl	Octane	SePro Corp.	Carmel, IN	http://www.sepro.com
Saflufenacil	Sharpen	BASF Corp.	Research Triangle Park, NC	http://agproducts.basf.us
Sulfentrazone	Dismiss	FMC Corp.	Philadelphia, PA	http://www.fmccrop.com

[a] Trade name listed here is the trade name of the products used in this study. Other trade names may exist for active ingredients listed in this table.

three trials each for spores and bulbils, respectively. Ten temperature main plots were created in a thermal-gradient table similar to that described by Hensley et al. (1982) set to range from 5 to 35 C. Plates were randomly assigned to a main plot. Actual temperatures in each lane were: 8.4, 15.9, 23.6, 28.7, 33.1, 33.7, 34.8, 34.9, 35.0, and 35.7 C and deviated 0.001% based on temperatures logged every second throughout the experiment.

Three Pioneer VI grow lights (Sunleaves Garden Products, Bloomington, IN) suspended at a height of 46 cm above the gradient table emitted approximately 200 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) for a 16-h photoperiod. Plates were photographed at initiation and every 7 d for 28 d for digital image analysis to determine germination percentage and average growth at each temperature. Digital images were taken with a Canon EOS Mark II (Canon Inc., Ohta-ku, Tokyo, Japan) on the following settings: F16, ISO 100, 2-s shutter speed, and white balance set to fluorescent lighting. The imaging platform consisted of an enclosure made of frosted acrylic for light diffusion placed inside a closed closet lit with fluorescent lamps to prevent interference from natural light sources. Images were analyzed for green color using a program written for Sigma Scan Pro 5.2 that optimizes green pixel detection (Karcher and Richardson 2003). Temperature responses were subjected to analysis of variance with sums of squares partitioned to reflect linear, quadratic, and lack of fit to linear and quadratic effects of temperature. Regressions were performed in SAS 9.2 (SAS Institute Inc, Cary, NC) only where analysis of various indicated a P value less than 0.05.

PRE Testing. Plates consisted of 4 mm MS medium plus Gamborg vitamins (MP Biomedicals LLC) solidified with 0.7% Phytagel (Sigma Chemical Co.). The medium was treated with 20 potential PRE products (Tables 1 and 2). Individual treatments were selected from products previously reported in the literature to control silvery threadmoss at least 70%, as well as compounds from similar classes of chemistry to those that have been reported to control moss; i.e., sulfentrazone was selected because carfentrazone had activity. Several products that had not been evaluated for silvery threadmoss control, such as methiozolin, were also included. Bulbils and spores were surface-sterilized and added to treated plates as

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Table 2. Percentage of reduction in area under the progress curve for green cover from silvery threadmoss bulbil and spore development over time. Spores and bulbils were grown at a constant 24 C and 16-h day in growth chambers at Glade Road Research Center, Blacksburg, VA, under each of the following treatments to evaluate reduction in growth over time.

Treatment	Rate	Rate	Bulbils	Spores	P value*
	g ai ha^{-1}	ppm	——% Reduction——		P = 0.05
Carf. + chloro. + fos.[a]	111 + 7,940 + 10,200	159 + 32 + 24	89.2	91.7	0.496
Sulfentrazone	140	95	87.4	92.4	0.581
Oxyfluorfen	538	19	87.0	86.3	0.917
Oxadiazon	1,700	9	86.4	92.2	0.099
Saflufenacil	25	0.15	86.0	83.1	0.613
Flumioxazin	224	0.88	85.9	91.8	0.083
Carf. + fosetyl-Al	111 + 10,200	159 + 24	83.9*	92.9*	0.007
Carfentrazone	111	159	83.8*	92.7*	0.012
Carf. + chloro.	111 + 7,940	159 + 32	83.4	91.0	0.125
Pyraflufen-ethyl	6	0.58	83.2	73.7	0.324
Chlorothalonil zinc	7,940	32	60.1*	92.8*	0.0002
Fosetyl-Al + prop.	10,200	24 + 6	50.8	17.9	0.165
Mancozeb + CuOH	1,910	25	41.3*	90.9*	< 0.0001
Fosamine	5,820	28	34.5	23.4	0.798
Phosphite	4,950	38	29.7	−96.7[c]	0.173
Fosetyl-Al (QP)	10,200	24	23.5	−113	0.149
Mineral oil	52,900	108	16.1	−65.2	0.320
Fosetyl-Al (Sig.)	10,200	24	3.50	−21.9	0.584
Methiozolin	1,500	28	−90.2*	17.3*	0.039
LSD[b]			32.8	103	

[a] Abbreviations: carf., carfentrazone; chloro., chlorothalonil zinc; fos., fosetyl-Al; prop., propiconazole; CuOH, copper hydroxide; Sig., Signature.

[b] Means within bulbil or spore were subjected to Fisher's protected LSD at the $\alpha = 0.05$ level.

[c] Negative values represent a growth response. Rather than causing a reduction in growth as expected, these products caused moss colonies to expand; therefore, percentage of reduction in area under the progress curve is negative for these products.

* Significant difference between spore and bulbil response for indicated treatment based on Fisher's protected LSD test at P = 0.05. These are individual comparisons between spore and bulbil response for a single treatment at a time.

previously described in methods for axenic culture. All plates were sealed with Parafilm (The Lab Depot) to prevent moisture loss over time. The experiments were arranged as randomized complete block designs with a 2 by 20 factorial treatment arrangement and four replications. Factor 1 was propagule (spore or bulbil) and factor 2 was chemical treatment (Tables 1 and 2). The study was conducted three times in space with each trial in a separate growth chamber. Growth chambers were set to a constant temperature of 24 C. A 16/8-h day/night cycle was used for these experiments and growth chambers received light from one eight-bulb Sun System Tek Light (Sunlight Supply, Inc. Vancouver, WA). Average PAR readings inside the chambers were 272 μmol m^{-2} s^{-1}.

Digital images of each plate were taken at 0, 7, 14, and 21 d after treatment as described above. The macro was optimized to evaluate the specific green coloration of silvery threadmoss protonemal and gametophyte growth resulting in settings for a hue range of 26 to 100 and a saturation of 0 to 75. Pixel counts at day 0 were considered 0% cover for a particular plate. Green pixel counts at day 7, 14, and 21 d were converted to percentage of cover and analyzed as increase in percentage of cover over time. Pixel counts over time were also summarized over dates to calculate area under the progress curve (AUPC) using the following equation, similar to other researchers evaluating increases in moss severity over time (Kennelly et al. 2010; Thompson et al. 2011):

$$\partial = \sum_{i=1}^{ni-1} \left((y_i + y_{(i-1)})/2(t_{(i-1)} - t_{(i)}) \right) \quad [1]$$

where ∂ represents AUPC, i is ordered sampling date, ni is the number of sampling dates, y is the proportion of green pixels detected, and t is time in days.

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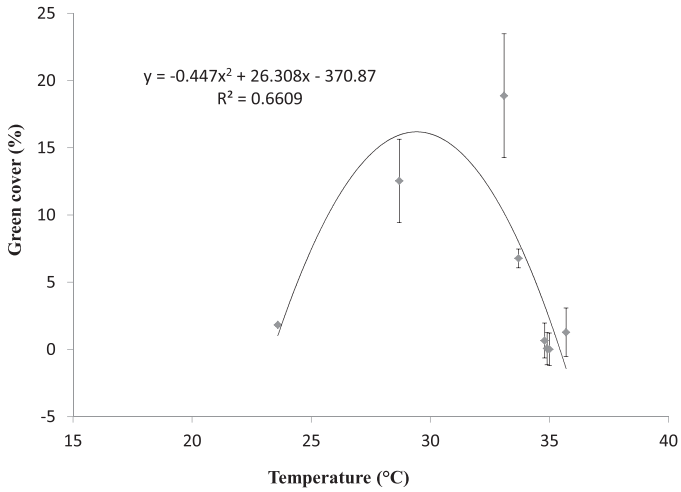

Figure 2. Temperature influence on moss protonemal cover from spores. Data points represent percentage of green cover over a petri plate after 21 d of growth at the given temperature. The fitted curve represents predicted values at given temperatures in between data points based on the equation shown. Error bars represent standard error.

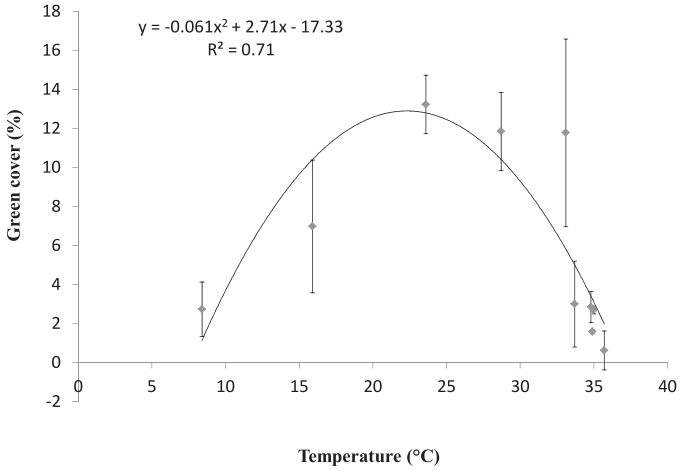

Figure 3. Temperature influence on moss gametophyte growth from bulbils. Data points represent percentage of green cover over a petri plate after 21 d of growth at the given temperature. The fitted curve represents predicted values at given temperatures in between data points based on the equation shown. Error bars represent standard error.

Data calculated using Equation 1 were then expressed as a percentage of reduction in AUPC compared to the nontreated check as follows:

$$\%\text{reduction} = \left(1 - (\partial_r/\partial_c)\right) \times 100 \qquad [2]$$

where ∂ represents AUPC, c represents the nontreated check, and r represents the treatment. Final data were arcsine square root transformed to improve normality based on the Shapiro-Wilk statistic using the NORMAL option in PROC UNIVARIATE in SAS 9.2 (SAS Institute Inc.). Transformed data were subjected to ANOVA using the GLM procedure in SAS 9.2 with sums of squares partitioned to reflect the herbicide-by-propagule factorial design and trials, which were considered random. Main effects and interactions were tested using mean square error associated with effect interactions with trial (McIntosh 1983). Means were separated with Fisher's protected LSD test at $\alpha = 0.05$. Interpretations of transformed and nontransformed data were identical; therefore, nontransformed means are presented for clarity.

Results and Discussion

Temperature Influence on Propagules. Trial interactions were not significant ($P > 0.05$) so data were pooled across trials. Attempts to fit both Gaussian and Lorentzian functions in proc NLIN in SAS 9.2 failed to converge for least squares determination, so a simple third-order polynomial equation was fit to growth data (Figure 2). Growth from spores began at approximately 20 C and continued to increase through 29.5 C with little to no growth evident at temperatures below 20 and above 34 C. An optimum temperature for spores was selected by identifying the point on the fitted curve where maximum growth is expected. Based on the curve spores grow optimally at 29.5 C (Figure 2). Duckett et al. (2004) reported most bryophytes can germinate and grow in vitro in a range of 5 to 25 C. Other studies suggest silvery threadmoss cultures will grow in sterile and nonsterile conditions at temperatures between 20 and 25 C, though none of these studies directly examined effects of temperature or indicated how the temperature was chosen (Buelovic et al. 2004; Horsley et al. 2011; Sabovljevic et al. 2010; Shaw and Albright 1990).

Gametophyte growth from bulbils started as low as 10 C and peaked at approximately 22.5 C when fit to a quadratic curve ($R^2 = 0.71$) (Figure 3). There is little literature examining moss bulbil or other vegetative propagule growth responses to temperature. Based on data analyzed from this temperature study, the propagule-by-treatment factorial PRE trials were executed at 24 C, a temperature that fell within the two optimal temperatures for spore (29.5

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C) and bulbil growth (22.5 C). Additionally, preparing the cultures on a solid medium more accurately represents field conditions where moss would initialize colonization in a soil substrate and begin to initiate shoot and rhizoid formation after a brief period of protonemal growth. Solid medium was selected for PRE screenings in lieu of earlier work performed in liquid culture (Liang et al. 2010).

PRE Screening. Trial interactions were not significant ($P > 0.05$) so data from all trials were pooled. For percentage of reduction in AUPC over time, there was a treatment-by-propagule interaction ($P = 0.013$) and data are presented by propagule (Table 2). The main effect of treatment was significant ($P < 0.0001$). The treatment-by-propagule interaction was likely due to a significant difference between bulbil and spore response to five treatments: carfentrazone, carfentrazone + fosetyl-Al, chlorothalonil zinc, mancozeb + copper hydroxide, and (methiozolin). For these five treatments, percentage of reduction in AUPC was greater when spores were treated than when bulbils were treated (Table 2). Methiozolin increased gametophyte growth from bulbils significantly compared to the nontreated (data not shown) and positively influenced AUPC for green cover over time (Table 2). No other treatment increased bulbil growth; however, several treatments increased AUPC for cover on spore plates, while methiozolin decreased AUPC for spore growth by 17% (Table 2). The products that increased protonemal growth from spores included two formulations of fosetyl-Al alone, phosphite, and mineral oil. It is unclear why these treatments significantly increased growth from spores.

Treatments that reduced AUPC for gametophyte growth from bulbils greater than 70% included carfentrazone, carfentrazone + fosetyl-Al, carfentrazone + chlorothalonil, carfentrazone + chlorothalonil + fosetyl-Al, sulfentrazone, oxyfluorfen, oxadiazon, saflufenacil, flumioxazin, and pyraflufen-ethyl (Table 2). Oxadiazon, oxyfluorfen, and flumioxazin have all been reported in the literature to provide effective (> 70%) POST or PRE moss control in containerized nursery ornamentals (Fausey 2003). Only oxadiazon has been reported for use on putting greens as a POST moss control option, reducing moss populations by as much as 50% 10 w (Burnell et al. 2004). The aforementioned products that reduced AUPC for gametophyte growth from bulbils by at least 70% also reduced protonemal growth from spores at similar or greater levels. Two additional products also reduced AUPC of spore protonemal growth by over 70%: chlorothalonil zinc and mancozeb + copper hydroxide (Table 2). An important result from this study is that the POST industry standard, carfentrazone, is also an effective PRE control product for silvery threadmoss spores and bulbils, controlling them 92.7 and 83.8%, respectively (Table 2). In addition to carfentrazone, chlorothalonil, mancozeb, oxadiazon, and pyraflufen-ethyl can be found in products labeled for use on creeping bentgrass putting greens. Other active ingredients would require supplemental labeling to be used on creeping bentgrass greens.

Products containing the active ingredient fosetyl-Al are labeled for use on greens for their fungicidal properties. Use of these products on silvery threadmoss–infested greens may exacerbate the problem, particularly when germinating from spores. However, programs utilizing carfentrazone as a tank-mixing partner may alleviate this trend. Methiozolin is a new product registered on creeping bentgrass putting greens in Japan and Korea for the control of annual bluegrass (*Poa annua* L.). Methiozolin is currently being evaluated under an experimental use permit for registration in the United States. Based on data presented here, methiozolin has the potential to increase silvery threadmoss pressure on putting greens.

Although phosphite fungicides and methiozolin increased silvery threadmoss growth under controlled environmental conditions future research should evaluate the impact of these products on silvery threadmoss populations on putting greens. Increases in both creeping bentgrass growth and vigor due to pest control in the field may subsequently lead to a reduction in silvery threadmoss due to improved turf competition. In previous studies Kennelly et al. (2010) found that increased height of cut and fertility improved moss suppression. Fungicide applications may also improve turf competitiveness with silvery threadmoss, and when proper cultural practices are utilized, such as nitrogen fertility and topdressing, moss encroachment can be minimized (Borst et al. 2010; Thompson et al. 2011). It is currently unknown to what extent silvery threadmoss population growth on putting greens is due to existing colony expansion vs. introduced propagule establishment. These data represent a first step at better understanding these processes and developing a more

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integrated approach to silvery threadmoss control. In addition, it will be important to evaluate PRE products with activity against silvery threadmoss propagules in the laboratory for their efficacy as PRE options in the field.

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