

Selective Broadleaf Weed Control in Turfgrass with the Bioherbicides *Phoma macrostoma* and Thaxtomin A

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Both regulatory and consumer forces have increased the demand for biopesticides, particularly in amenity areas such as turfgrass. Unfortunately, few natural products are available for selective weed control in turfgrass. Two bioherbicides reported to control broadleaf weeds without injuring turfgrass are *Phoma macrostoma* and thaxtomin A. Field and container experiments were conducted to evaluate PRE and POST efficacy of *P. macrostoma* and thaxtomin A on regionally important broadleaf weeds. In container experiments, PRE applications of *P. macrostoma* provided 65 to 100% control of dandelion, marsh yellowcress, and flexuous bittercress, equivalent to that of pendimethalin. Control of yellow woodsorrel, henbit, hairy galinsoga, common chickweed, or annual bluegrass was less than with pendimethalin. In contrast, POST applications did not control any species as well as an industry-standard synthetic auxin herbicide. PRE or POST applications of thaxtomin A controlled six of the eight species tested as well as the industry-standard PRE or POST herbicides. In field tests, overall PRE broadleaf weed control with *P. macrostoma* and thaxtomin A peaked 4 wk after treatment at 64 and 72%, respectively, and declined afterward, suggesting that these bioherbicides possess short residuals and therefore must be reapplied for season-long control. Overall POST broadleaf weed control using *P. macrostoma* and thaxtomin A was only 41 and 25%, respectively. PRE followed by early-POST applications of thaxtomin A provided $\geq 86\%$ henbit control. These results suggest that both *P. macrostoma* and thaxtomin A are capable of controlling certain broadleaf weeds in turfgrass. However, both lack efficacy on some important weed species, particularly chickweed. Thaxtomin A efficacy on henbit was improved by increased dose and by PRE followed by early-POST applications.

Nomenclature: *Phoma macrostoma*; thaxtomin A; hairy galinsoga (*Galinsoga quadriradiata*); dandelion, *Taraxacum officinale* G.H. Weber ex Wiggers, TAROF; yellow woodsorrel, *Oxalis stricta* L., OXAST; marsh yellowcress, *Rorippa palustris* (L.) Bess., RORIS; ivyleaf speedwell, *Veronica hederifolia* L., VERHE; annual bluegrass (*Poa annua*); flexuous bittercress, *Cardamine flexuosa* With., CARFL; henbit, *Lamium amplexicaule* L., LAMAM; common chickweed, *Stellaria media* (L.) Vill., STEME; large hop clover, *Trifolium campestre* Schreb., TRFCA; sparrow vetch, *Vicia tetrasperma* (L.) Schreb., VICTE; field madder, *Sherardia arvensis* L., SHRAR; tall fescue, *Lolium arundinaceum* (Schreb.) S.J. Darbyshire, 'The Rebels', 'Top Choice'.

Key words: Biocontrol, biological weed control, biopesticide, macrocidin.

Tanto fuerzas regulatorias como los consumidores han incrementado la demanda por biopesticidas, particularmente en áreas amenas tales como áreas con césped. Desafortunadamente, hay pocos productos naturales disponibles para el control selectivo de malezas en céspedes. Dos bioherbicidas reportados para el control de malezas de hoja ancha sin causar daño al césped son *Phoma macrostoma* y thaxtomin A. Experimentos de campo y en contenedores fueron realizados para evaluar la eficacia de *P. macrostoma* y thaxtomin A PRE y POST en malezas de hoja ancha importantes en la región. En experimentos con contenedores, las aplicaciones PRE de *P. macrostoma* brindó 65 a 100% de control de *Taraxacum officinale*, *Rorippa palustris*, y *Cardamine flexuosa*, el cual fue equivalente al control con pendimethalin. El control de *Oxalis stricta*, *Lamium amplexicaule*, *Galinsoga quadriradiata*, *Stellaria media*, o *Poa annua* fue menor que con pendimethalin. En contraste, las aplicaciones POST no controlaron ninguna de las especies tan bien como un herbicida sintético auxinic estándar en la industria. Las aplicaciones PRE o POST de thaxtomin A controlaron seis de las ocho especies evaluadas tan bien como los herbicidas PRE y POST estándar en la industria. En los ensayos de campo, en general el control PRE de malezas de hoja ancha con *P. macrostoma* y thaxtomin A alcanzó el máximo nivel 4 semanas después del tratamiento con 64 y 72%, respectivamente, y declinó después de este momento, sugiriendo que estos bioherbicidas poseen un corto efecto residual, por lo que deben ser replicados para obtener control a lo largo de toda la temporada. En general, el control POST de malezas de hoja ancha usando *P. macrostoma* y thaxtomin A fue solamente 41 y 25%, respectivamente. Aplicaciones PRE seguidas por POST-tempranas de thaxtomin A brindaron $\geq 86\%$ de control de *L. amplexicaule*. Estos resultados sugieren

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que *P. macrostoma* y thaxtomin A son capaces de controlar algunas malezas de hoja ancha en céspedes. Sin embargo, ambos carecen de eficacia en el control de algunas especies de malezas importantes, particularmente *S. media*. La eficacia de thaxtomin A en *L. amplexicaule* fue mejorada al aumentar la dosis y al hacer aplicaciones PRE seguidas de aplicaciones POST-tempranas.

In the United States, turfgrass occupies more than 12 million ha of land, including 50 million lawns, plus golf courses, athletic fields, parks, and sod farms (Grewal 1999). In total, managed turfgrass areas represent roughly 23% of developed land in the United States (Hernke and Podein 2011). Turfgrass managers aim to maintain healthy, homogenous, and weed-free turfgrass to improve aesthetics, reduce human health risks associated with allergies, and, in the case of golf courses and athletic fields, ensure a uniform playing surface (Zimdahl 2013). Weed control in turfgrass is heavily dependent on synthetic chemical herbicides. Grube et al. (2011) estimated that roughly 45 million kg of synthetic herbicides were applied to noncrop areas in the United States between 2000 and 2007. In 1996 over \$200 million was spent on synthetic herbicides for use in highly maintained turfgrass in the United States, with sales predicted to increase 2 to 4% annually (Porpiglia et al. 1996).

The widespread use of synthetic pesticides in residential lawns has generated controversy in recent years, and regulatory agencies in several regions of North America have imposed significant restrictions on these practices. The governments of Quebec and Ontario enacted bans on the use of pesticides in landscapes in 2003 and 2009, respectively (Belair et al. 2010). In the United States similar restrictions have been imposed by state and local governments. For example, the State of New York's Child Safe Playing Field Act banned the use of synthetic chemical pesticides on school properties (Grant 2011). Such restrictions have heightened the need for alternative weed management strategies in turfgrass.

Biological control and biopesticides are alternatives to synthetic herbicides that have received considerable attention in recent years. The global biopesticide market has grown in recent years and is expected to reach \$4.17 billion by 2023 (Anonymous 2016); yet, bioherbicides make up only a small portion of the total market (Bailey et al. 2010). Developing successful bioherbicides has proven challenging, and past bioherbicides have

frequently offered a narrow spectrum of control. Additionally, their effectiveness could be significantly affected by environmental conditions at the time of application, limiting their commercial potential (Chandler et al. 2011; Harding and Raizada 2015).

There are currently few biologically based weed control options available to turfgrass managers in North America. Corn gluten meal, a byproduct of the corn milling process, has been reported to provide PRE control of weedy summer annual grasses (Liu et al. 1994; McDade and Christians 2000). However, in more recent studies, corn gluten improved turfgrass quality but did not provide commercially acceptable levels of weed control (Siva 2014; St. John and DeMuro 2013). Hence more effective bioherbicide options for PRE control of weeds are needed.

Several bioherbicides have been reported to have potential for POST broadleaf weed control in turfgrass. Iron hydroxyethylenediaminetetra-acetic acid (FeHEDTA), a chelated iron formulation, was registered by the U.S. Environmental Protection Agency as a bioherbicide (Anonymous 2009). FeHEDTA has been demonstrated to selectively control a large number of broadleaf weeds when applied POST, causing rapid foliar necrosis of target weeds without injury to turfgrass (Wilén 2012). However, because of the synthetic chelating agent, it is not an option in certified organic systems. *Sclerotinia minor*, a plant pathogen that has been evaluated as a potential biological control agent for broadleaf weeds in turfgrass, is currently labeled for use in Canada but is not yet registered in the United States (Riddle et al. 1991; Schnick et al. 2002). Like many past bioherbicides that have struggled to achieve commercial success, the efficacy of *S. minor* can vary greatly depending on environmental factors such as moisture and temperature, presenting an obstacle for turfgrass managers attempting to control weeds organically (Abu-Dieyeh and Watson 2007; Bourdôt et al. 2011; Siva 2014). Turfgrass managers who wish to

provide organic lawn care alternatives for weed control.

Two other biologically based products reported to have potential for control of broadleaf weeds in turfgrass are *Phoma macrostoma* and thaxtomin A. *Phoma macrostoma*, a coelomycete fungal pathogen isolated from Canada thistle (*Cirsium arvense*), has been shown to cause bleaching, chlorosis, and eventual death in a variety of broadleaf weed species when applied both PRE and POST (Bailey et al. 2009; Evans et al. 2013). The fungus is nonpathogenic to monocots but has been shown to infect 38 economically important dicotyledonous weed species from 12 families (Bailey et al. 2011). Because of the pathogen's ability to control broadleaf weeds without harming grass species, commercial applications in the turfgrass industry are currently being explored. However, research on *P. macrostoma* has been conducted almost exclusively in Canada. Data from growth-chamber studies demonstrated that the pathogen remains infectious even at higher temperatures, suggesting that warmer environmental conditions would not limit the efficacy of *P. macrostoma* (Neal et al. 2013b). However, no data are currently available on *P. macrostoma* efficacy under field conditions in warmer climates such as the southern United States.

Thaxtomin A, a chemical produced by the bacterium *Streptomyces scabies*, has been reported to have phytotoxic activity similar to that of cellulose biosynthesis inhibitors, causing seedling stunting, cellular hypertrophy, and cell wall lignification in susceptible species (Fry and Loria 2002; King et al. 1992). King et al. (2001) demonstrated that thaxtomin A is nonsystemic but potentially highly phytotoxic. Their data also suggested that thaxtomin A has more activity on dicotyledonous species than on monocots and is more active when applied PRE than POST, though symptoms were observed at both application timings. Although these initial screenings identified thaxtomin A as having promise as a bioherbicide in turfgrass, further research into the spectrum of weeds controlled by thaxtomin A and its response to the varied environmental conditions present in typical turfgrass systems is necessary before commercialization.

Although both *P. macrostoma* and thaxtomin A have potential as bioherbicides, more research into their ability to effectively control a large number of

common weed species is required. Furthermore, past research has demonstrated that bioherbicides that perform well in lab and greenhouse-based screenings or under ideal environmental conditions in field experiments may not provide similar results under varied conditions in a commercial setting. The objectives of this research were to (1) evaluate the potential of PRE and POST applications of *P. macrostoma* and thaxtomin A to control common weeds of turfgrass in the southern United States in container screenings, (2) determine product efficacy in newly seeded turfgrass in field studies, and (3) evaluate the potential for enhancing the efficacy of thaxtomin A on poorly controlled species with multiple applications and increased dose.

Materials and Methods

Common Methods. All experiments were conducted at the North Carolina State University Horticultural Field Lab in Raleigh, NC (35.79°N, 78.7°W). The soil type on this site was a Cecil gravelly sandy loam (fine, kaolinitic, thermic Typic Kanhapludults) with 2.87% humic matter and a pH of 6.8. All treated plots were 1 m² and, unless otherwise noted, were arranged in randomized complete block designs with four replicates. Unless otherwise noted, application methods were similar in all experiments. Granular treatments were applied in preweighed aliquots using a handheld shaker jar. Spray treatments were applied using a CO₂-pressurized backpack sprayer equipped with two 8004 flat fan nozzles (TeeJet, Spraying Systems Co., North Ave. and Schmale Rd., Wheaton, IL 60187) calibrated to deliver 280 L ha⁻¹. Weed control was visually evaluated on a 0-to-10 scale, similar to that described in Frans et al. (1986), where 0 = no control (weed populations and cover were equal to the nontreated areas) and 10 = 100% control (no living weed biomass present). Intermediate values are visual estimates of percent reduction in aboveground plant biomass compared with the nontreated. These ratings were converted to a percent scale for presentation. In container experiments, aboveground fresh weights were recorded in the first repetition of each experiment. Fresh weights and visual ratings were negatively correlated, ($R < -0.84$); thus only visual ratings were conducted thereafter. In field experiments, weed

counts and percent ground cover were also documented and are described below.

PRE Weed Control. *PRE Weed Control Screening in Containers.* One-liter pots were filled with a hammer-milled pine bark nursery substrate (Parker Bark Company, 3295 U.S. Hwy. 117, Rose Hill, NC 28458) amended with 4.75 kg m^{-3} of a controlled-release granular fertilizer (18–4–8 w/ minors 8–9mo[®], Harrell's Fertilizer Inc., 5105 New Tampa Highway, Lakeland, FL 33815), then hand watered to settle the substrate. Weeds were surface seeded on September 13, 2012 at a rate of about 30 germinable seeds per pot, each species in separate pots. The test was repeated beginning September 16, 2013. Weed species were: yellow woodsorrel, henbit, marsh yellowcress, flexuous bittercress, hairy galinsoga, dandelion, common chickweed, ivyleaf speedwell, and annual bluegrass. Seeds of all weed species were locally collected in previous years. After seeding pots were overhead irrigated to settle the seeds and substrate, then treatments were applied.

Treatments included a nontreated control, thaxtomin A at 190 or 380 g ai ha⁻¹, and three doses of *Phoma macrostoma*. Thaxtomin A was a liquid formulation containing about 10 g ai L⁻¹ (MBI-005, Marrone Bio Innovations Inc., 1540 Drew Ave., Davis, CA 95618). *Phoma macrostoma* was formulated as dry granules containing approximately 400 macrocidin units (mu) g⁻¹ (a proprietary standardized measure of active ingredient content) and was applied at rates of 3,250, 6,500, or 13,000 mu m⁻² (Bailey et al. 2011). Two industry-standard PRE herbicide treatments were included: corn gluten meal (Preen[®] Organic Weed Preventer, Lebanon Seaboard Corporation, 1600 East Cumberland St., Lebanon, PA 17042) at 97 kg ha⁻¹, and pendimethalin (Pendulum[®] 2G, BASF Corporation, 26 Davis Dr., Research Triangle Park, NC 27709) at 3,300 g ai ha⁻¹. After applications were made, pots were maintained on an outdoor nursery pad and received 0.5 cm of overhead irrigation three times per day.

The experiment was arranged in a randomized complete block design with five replications in 2012 and six replications in 2013 (each experimental unit contained a pot of each weed species). Weed control was visually evaluated 3, 4, 5, and 6 wk after treatment as described above.

PRE Weed Control in Seedling Tall Fescue Turfgrass. Field experiments were initiated on September 26, 2012 and September 9, 2013. In both years, a trial location with a history of winter annual broadleaf weed infestations was treated with glyphosate 2 wk before seeding. Debris was removed, and the sites were raked smooth, core cultivated, and amended with a 9–13–7 (N–P₂O₅–K₂O) fertilizer (Fertilo-me New Lawn Starter Fertilizer[®], Voluntary Purchasing Group, 230 FM 87, Bonham, TX 75418) at a rate of 24.4 kg N ha⁻¹. In 2012, the trial site was seeded with 'The Rebels'[®] tall fescue blend (Pennington Seeds, Inc., 1280 Atlanta Hwy., Madison, GA 30650), and in 2013 the trial site was seeded with 'Top Choice'[®] tall fescue blend (Mountain View Seeds, 8955 Sunnyview Rd. NE, Salem, OR 97305). In both years, turfgrass was seeded at a rate of 195 kg ha⁻¹ using a rotary spreader. After seeding, trial areas were irrigated as needed to promote establishment. Both sites received a second fertilization with the same fertilizer at a rate of 24.4 kg N ha⁻¹ 4 wk after seeding. After establishment, trial areas were mowed as needed at a 7.6-cm height with a rotary mower, with clippings returned to the site. The areas received supplemental irrigation as needed to prevent drought stress.

Initial PRE treatments were applied at seeding on September 27, 2012 and September 9, 2013, with retreatments on November 16, 2012 and October 9, 2013. PRE treatments included thaxtomin A at 190 or 380 g ai ha⁻¹, *P. macrostoma* at 6,500 or 13,000 mu m⁻², corn gluten meal at 97 kg ha⁻¹, and a nontreated control. Pendimethalin applied before seeding is known to prevent turfgrass establishment (Keeley and Zhou 2005, Neal and Osmeloski 1992) and was therefore not included in this experiment. Thaxtomin A was applied using a CO₂-pressurized backpack sprayer equipped with flat-fan nozzles and calibrated to deliver 467 L ha⁻¹ in 2012 or 280 L ha⁻¹ in 2013. Simultaneous experiments in 2012 had demonstrated no differences in thaxtomin A efficacy at these two carrier volumes (Neal et al. 2013a).

Weed control was evaluated weekly for 8 wk following treatment, after which low temperatures inhibited further weed germination. Control of each weed species present was evaluated visually as described above. Four weeks after treatment (WAT), weed counts were conducted for each

species uniformly distributed in the field. A final evaluation was conducted the following spring, approximately 32 wk after initial treatments, to evaluate the long-term impact of PRE treatments on weed populations.

POST Weed Control. *POST Weed Control in Containers.* Methods used in these experiments were identical to those used in the PRE container experiment described above, except for the following. In the POST efficacy experiment, treatments were applied October 16, 2012 and October 18, 2013, approximately 4 wk after surface-seeding weeds. By that time, weeds had reached an average height of 2 to 5 cm. Treatments were reapplied 3 wk later, on November 6, 2012 and November 6, 2013. Treatments in the POST experiment included a nontreated control, thaxtomin A at 190 or 380 g ai ha⁻¹, *P. macrostoma* at 3,250, 6,500, or 13,000 mu m⁻², and a ready-to-use (RTU) auxin herbicide spray (Weed B Gon MAX RTU®, 0.05% dimethylamine salt of dicamba, 0.12% dimethylamine salt of 2,4-dichlorophenoxyacetic acid, 0.22% dimethylamine salt of methylchlorophenoxypropionic acid; The ORTHO Group, 1411 Scottslawn Rd., Marysville, OH 43040) applied at approximately 1,250 g ae ha⁻¹ (containing 400 g ae ha⁻¹ 2,4-D plus 702 g ae ha⁻¹ mecoprop and 148 g ae ha⁻¹ dicamba). After treatment, pots were placed in a nonheated shade house covered with clear plastic to protect seedlings from frost and received overhead irrigation as needed.

POST Weed Control in Seedling Tall Fescue Turf. Methods used in this experiment were identical to those used in the PRE weed control in seedling tall fescue experiment described above, except for the following. POST treatments were applied 7 to 8 wk after turfgrass seeding, on November 16, 2012 and November 4, 2013. Treatments included a nontreated control, thaxtomin A at 190 or 380 g ai ha⁻¹, *P. macrostoma* at 6,500 or 13,000 MU m⁻², and Weed B Gon MAX RTU at 1,250 g ae ha⁻¹. At the time POST treatments were applied, dandelion, common chickweed, henbit, and sparrow vetch were the most common weed species present. Weed control was visually evaluated 1, 2, and 4 WAT. After that time, plants were dormant because of cold temperatures.

Enhancing Thaxtomin A Activity on Poorly Controlled Species. Two strategies for enhancing

thaxtomin A efficacy on poorly controlled species were compared. Henbit was chosen as a model species because it was susceptible but not consistently well controlled PRE or POST by thaxtomin A in the container and newly seeded turfgrass studies described above. Experiments were conducted in planting beds with a history of heavy henbit infestations.

POST Control of Henbit with Increased Dose. Treatments in this experiment included thaxtomin A at 190, 380, or 570 g ha⁻¹ and a nontreated control. Field beds were cultivated in early September of each year to stimulate winter annual weed germination. Treatments were applied on October 25, 2013, and the experiment was repeated beginning October 24, 2014. A natural population of henbit was present in all plots at the time of application, averaging 70 to 80% cover. Growth stages were from cotyledon to four leaf pairs with an average height of 7.5 cm. Weed control was evaluated weekly for 17 WAT as described above. Visual estimations of percent weed cover were also recorded. Thaxtomin A dose response was subjected to linear regression analysis using Proc REG in SAS 9.4 (SAS 9.4, SAS Institute, Inc., 100 SAS Campus Dr., Cary, NC 27513).

PRE Followed by Early POST Control of Henbit. In both years, field plots were established in a recently tilled planting bed with a history of heavy henbit infestation. Treatments in this experiment included thaxtomin A at 190 or 380 g ha⁻¹, pendimethalin at the labeled rate of 3,300 g ha⁻¹, and a nontreated control. Thaxtomin A and pendimethalin were applied PRE on September 20, 2013 and on October 2, 2014. Thaxtomin A then was reapplied early POST, 4 wk after initial applications, on October 18, 2013 and October 30, 2014. At the time of the second applications, henbit seedlings with two to four leaf pairs were present in nontreated plots, whereas henbit was present at lower populations in treated plots and varied from cotyledons to two leaf pairs. Weed control was visually evaluated as previously described, and percent ground cover of henbit was estimated. Weekly evaluations were initiated 4 wk after the first application.

Statistical Analysis. Data collected from all experiments were subjected to ANOVA using Proc GLM in SAS 9.4 to test for both main treatment

Table 1. PRE control of yellow woodsorrel, henbit, marsh yellowcress, and flexuous bittercress in containers using bioherbicides 28 d after application. Research conducted at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

Herbicide	Rate	Units ^a	Yellow woodsorrel		Henbit		Marsh yellowcress		Flexuous bittercress	
			2012	2013	2012	2013	2012	2013	2012	2013
			% control ^b							
<i>Phoma macrostoma</i>	3,250	mu m ⁻²	10	15	3	17	90	37	36	27
<i>P. macrostoma</i>	6,500	mu m ⁻²	8	15	20	7	100	53	58	25
<i>P. macrostoma</i>	13,000	mu m ⁻²	58	15	63	27	100	87	95	65
Thaxtomin A	190	g ai ha ⁻¹	86	22	63	32	78	38	12	45
Thaxtomin A	380	g ai ha ⁻¹	100	60	93	30	100	37	60	37
Corn gluten	97	kg ha ⁻¹	8	38	15	23	6	25	0	13
Pendimethalin	3,300	g ai ha ⁻¹	100	100	100	98	98	98	83	85
LSD _{0.05}			24	27	25	31	12	36	19	26

^a Abbreviation: mu, macrocidin units.

^b Percent control based on visual estimations of reductions in aboveground plant biomass, where 0 = no reduction and 100 = no weeds present.

effects and interactions. Where treatment effects were determined to be significant, treatment means were separated using Fisher's protected LSD method at a significance level of 0.05. When the interaction between year and treatment effect was not significant, data were pooled across years. When there was a significant interaction between years, data are presented by year.

Results and Discussion

PRE Weed Control. *PRE Weed Control in Containers.* Corn gluten meal provided little or no control on most weed species tested in these experiments (Tables 1 and 2). Both *P. macrostoma* and thaxtomin A controlled some weed species equivalent to pendimethalin, and percent weed control increased with increasing dose. Treatment-by-year interactions were significant for all species; thus data are presented by year.

Weed control with *P. macrostoma* was lower in 2013 than in 2012. In 2012, *P. macrostoma* at 3,250 and 6,500 mu m⁻² provided $\geq 90\%$ control of marsh yellowcress and dandelion, but $\leq 58\%$ control of all other species (Tables 1 and 2). In 2013, control of all weed species at these rates was $\leq 58\%$. At 13,000 MU m⁻², *P. macrostoma* provided control of marsh yellowcress, flexuous bittercress, and dandelion equal to pendimethalin in both years (Tables 1 and 2). Control of henbit, common chickweed, annual bluegrass, and yellow woodsorrel was less than that of pendimethalin in both years (Tables 1 and 2). Hairy galinsoga was

not well controlled by *P. macrostoma* or pendimethalin in either year.

In the 2012 experiment, thaxtomin A at 190 g ai ha⁻¹ controlled yellow woodsorrel and annual bluegrass 86 and 93%, respectively, equivalent to pendimethalin (Tables 1 and 2). Control of all other species was less than pendimethalin. Thaxtomin A at 380 g ai ha⁻¹ provided $\geq 93\%$ control of yellow woodsorrel, henbit, marsh yellowcress, dandelion, and annual bluegrass, as well as 75% control of hairy galinsoga, all equivalent to pendimethalin (Tables 1 and 2). Thaxtomin A at 380 g ai ha⁻¹ provided 60% control of flexuous bittercress and 20% control of common chickweed, both less than pendimethalin (Tables 1 and 2).

PRE weed control with thaxtomin A was consistently lower in 2013 than in 2012. In 2013, thaxtomin A at 190 g ai ha⁻¹ provided $\leq 45\%$ control of all species tested. At 380 g ai ha⁻¹, thaxtomin A provided $\leq 60\%$ control of all species in 2013.

The reasons for differences in thaxtomin A and *P. macrostoma* efficacies between 2012 and 2013 are unclear. Efficacies in POST container experiments were similar both years. Thaxtomin A product samples from each year were compared in a bioassay with no differences observed (data not shown), eliminating differences in bioactivity of that product as a causative factor. Application methods were identical between years. Temperatures over the course of the experiment were similar, averaging 21.7 C in 2012 and 21.5 C in 2013. The week after treatment, the site received 7.9 cm of rainfall in 2012 and 3.5 cm in 2013, and differences in rainfall

Table 2. PRE control of hairy galinsoga, dandelion, common chickweed, and annual bluegrass in containers using bioherbicides 28 d after application. Research conducted at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

Herbicide	Rate	Units ^a	Hairy galinsoga		Dandelion		Common chickweed		Annual bluegrass	
			2012	2013	2012	2013	2012	2013	2012	2013
			% control ^b							
<i>Phoma macrostoma</i>	3,250	mu m ⁻²	6	13	90	32	4	18	26	10
<i>P. macrostoma</i>	6,500	mu m ⁻²	16	5	100	58	10	7	12	5
<i>P. macrostoma</i>	13,000	mu m ⁻²	16	32	100	100	2	23	6	7
Thaxtomin A	190	g ai ha ⁻¹	18	32	78	27	6	25	93	30
Thaxtomin A	380	g ai ha ⁻¹	75	27	100	15	20	3	100	47
Corn gluten	97	kg ha ⁻¹	24	12	6	7	0	7	16	0
Pendimethalin	3,300	g ai ha ⁻¹	58	40	98	42	96	75	94	98
LSD _{0.05}			23	25	19	23	13	20	12	17

^a Abbreviation: mu, macrocidin units.

^b Percent control based on visual estimations of reductions in aboveground plant biomass compared with nontreated plots, where 0 = no reduction and 100 = no weeds present.

between years may be significant. Hoskins et al. (2014) demonstrated that solute transport through pine bark substrates is more rapid under unsaturated conditions. However, because pots received an additional 1.5 cm of irrigation per day throughout the experiment, differences in rainfall are unlikely to have been a major factor in these results. Differences in substrate between 2012 and 2013 may have also played a part. Although both experiments were conducted in 100% hammer-milled pine bark, such substrates can differ greatly in their textural and physical properties. These factors have been shown to have an impact on persistence and leaching of herbicides in pine bark substrates (Fenoll et al. 2014; Wehtje et al. 2012), and it is possible that textural and compositional differences may have affected the residual activity of the bioherbicides tested. Substrate pH also varied between years, measuring 5.6 in 2012 and 3.8 in 2013. Differences in soil pH have been shown to affect the persistence and phytotoxicity of certain herbicides (Grey & McCullough 2012; Rosenkrantz et al. 2013). However, no differences in thaxtomin A PRE efficacy were observed in a bioassay comparing pine bark substrates with pH adjusted to 4.0 or 5.8 (data not shown). Consequently, further exploration into the influence of environmental factors on the efficacy and persistence of these biopesticides is needed.

PRE Weed Control in Seedling Tall Fescue Turf. Percent control ratings were negatively correlated with individual-species weed counts ($R = -0.77$ and

-0.81 in 2012 and 2013, respectively); therefore, only percent control data are presented. Treatment-by-year interactions were nonsignificant for all species; therefore, data were pooled across years for presentation.

Similar to the container experiment, corn gluten meal did not control broadleaf weeds when evaluated 4 WAT (Table 3). Control improved later in the season, with 39% broadleaf weed control observed 32 WAT. In general, control of most species increased with increased dose of *P. macrostoma* or thaxtomin A.

Phoma macrostoma at 6,500 mu m⁻² controlled dandelion 87%, but other species were controlled $\leq 19\%$ (Table 3). At 13,000 mu m⁻², *P. macrostoma* provided 64% control of henbit and 99% control of dandelion but $\leq 28\%$ control of common chickweed and field madder (Table 3). Sixty-four percent and 61% control of broadleaf weeds was observed 4 WAT and 10 WAT, respectively, by 13,000 MU m⁻² of *P. macrostoma*. Overall broadleaf weed control was 42 to 50% the following spring, 32 WAT, with no difference between doses of *P. macrostoma* (Table 3).

Thaxtomin A at 190 g ha⁻¹ provided 54% broadleaf weed control 4 WAT and 21% 32 WAT (Table 3). At 380 g ha⁻¹, thaxtomin A provided $\geq 60\%$ control of henbit, dandelion, and field madder, but only 10% control of common chickweed (Table 3). Thaxtomin A at 380 g ha⁻¹ provided 72% overall broadleaf weed control 4 WAT, but control had declined to 54% 10 WAT and was only 26% 32 WAT.

Table 3. PRE control of henbit, dandelion, common chickweed, field madder, and overall broadleaf weeds and injury to newly seeded tall fescue turf using bioherbicides. Research conducted at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

Herbicide	Rate	Units	Weed control, 4 WAT ^{a,b}				Combined broadleaf weeds ^c			Tall fescue,
			Henbit	Dandelion	Common chickweed	Field madder	4 WAT	10 WAT	32 WAT	10 WAT
			— % control —						% cover ^d	
Nontreated	n/a	n/a	—	—	—	—	—	—	—	72
<i>Phoma macrostoma</i>	6,500	mu m ⁻²	19	87	13	12	27	24	50	72
<i>P. macrostoma</i>	13,000	mu m ⁻²	64	99	9	28	64	61	42	76
Thaxtomin A	190	g ai ha ⁻¹	51	21	9	41	54	47	21	51
Thaxtomin A	380	g ai ha ⁻¹	70	61	10	60	72	54	26	38
Corn gluten	97	kg ha ⁻¹	9	24	9	30	6	20	39	76
LSD _{0.05}			34	23	19	34	17	19	21	13

^a Percent control based on visual estimations of reductions in aboveground weed coverage, where 0 = no reduction and 100 = no weeds present. Data pooled across years, as treatment-by-year interaction was not significant. Visual ratings were relative to the nontreated plots; therefore, data for the nontreated were omitted from those analyses.

^b Abbreviations: mu, macrocidin units; WAT, weeks after treatment; n/a, not applicable.

^c Combined broadleaf weeds is an estimate of percent weed control for all broadleaf weeds in the treated area and includes those evaluated individually and any other dicot weeds present.

^d Visual estimates of % turfgrass coverage. Data pooled across years, as treatment-by-year interaction was not significant.

Tall fescue establishment was not affected by *P. macrostoma* or corn gluten treatments but was significantly hindered by both rates of thaxtomin A. Ten WAT, turfgrass cover in nontreated plots was 72%, whereas turfgrass cover in plots treated with thaxtomin A at 190 and 380 g ai ha⁻¹ was 58 and 31%, respectively (Table 3). These findings suggest that thaxtomin A and *P. macrostoma* have the potential to control some broadleaf weeds when applied PRE, but thaxtomin A will reduce tall fescue cover when applied at seeding. Overall, none of the bioherbicides tested provided effective control of all weed species present in the experiment.

POST Weed Control. *POST Weed Control in Containers.* Treatment-by-year interactions were significant for henbit, marsh yellowcress, flexuous bittercress, dandelion, and common chickweed; therefore, data for these species are presented by year. Data for yellow woodsorrel, hairy galinsoga, and ivyleaf speedwell are pooled across years. Injury symptoms caused by thaxtomin A and the synthetic auxin herbicide developed slowly and did not become fully apparent until several weeks after application; therefore, only data from the final evaluation, 6 wk after the second treatment, are presented.

The industry-standard auxinic herbicide, Weed B Gon RTU, provided similar control of most species in 2012 and 2013 (79 to 100%). The exception was henbit, which was controlled 24% in 2012 and 82% in 2013. In contrast, *P. macrostoma* was not effective when applied POST, controlling all species ≤ 54% in both years regardless of application rate. No *P. macrostoma* treatment controlled any species in the experiment equal to the auxinic herbicide (Tables 4 and 5). Symptoms observed on weeds treated with *P. macrostoma* were consistent with those reported by Bailey et al. (2009) and included bleaching and chlorosis. However, most species displayed chlorosis shortly after treatment but eventually recovered.

POST control of broadleaf weeds was improved by increasing thaxtomin A dose from 190 to 380 g ai ha⁻¹ (Tables 4 and 5). Thaxtomin A at 380 g ai ha⁻¹ provided ≥ 80% control of henbit, yellow woodsorrel, hairy galinsoga, and ivyleaf speedwell equal to or better than the auxinic herbicide (Tables 4 and 5). In 2012, control of marsh yellowcress, flexuous bittercress, dandelion, and common chickweed was ≤ 74% and less than the auxinic herbicide (Table 5). Control of those species with thaxtomin A at 380 g ha⁻¹ was better in 2013, with ≥ 95% control of marsh yellowcress and flexuous

Table 4. POST control of container-grown yellow woodsorrel, hairy galinsoga, and ivyleaf speedwell using bioherbicides, 42 d after two applications. Research conducted at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

Herbicide	Rate	Units ^a	% control ^b		
			Yellow woodsorrel	Hairy galinsoga	Ivyleaf speedwell
<i>Phoma macrostoma</i>	3,250	mu m ⁻²	20	5	0
<i>P. macrostoma</i>	6,500	mu m ⁻²	13	10	3
<i>P. macrostoma</i>	13,000	mu m ⁻²	12	9	5
Thaxtomin A	190	g ai ha ⁻¹	79	60	82
Thaxtomin A	380	g ai ha ⁻¹	98	80	93
Weed B Gon ^c	1,250	g ae ha ⁻¹	95	87	79
LSD _{0.05}			9	8	7

^a Abbreviation: mu, macrocidin units.

^b Percent control based on visual estimations of reductions in aboveground plant biomass compared with nontreated plots, where 0 = no reduction and 100 = complete plant mortality. Data pooled across years, as treatment-by-year effect was not significant.

^c Ready-to-use formulation of auxinic herbicides containing 0.026% dicamba, 0.605% 2,4-dichlorophenoxyacetic acid, and 0.149% methylchlorophenoxypropionic acid.

bittercress observed. Dandelion and chickweed control was better in 2013 than in 2012 but was not equal to the auxinic herbicide (Table 5). Injury symptoms on plants treated with thaxtomin A included seedling stunting and warping of the growing points, common symptoms of cellulose biosynthesis inhibition, similar to those described by King et al. (2001).

POST Weed Control in Seedling Tall Fescue Turf. Treatment-by-year interactions were significant for common chickweed, sparrow vetch, and large hop

clover; therefore, data for those species are presented by year. For all other species, treatment-by-year interactions were not significant and data were pooled across years.

Phoma macrostoma provided ≤ 10% control of common chickweed in both years when applied POST (Table 6), consistent with results from the container experiment. In 2012, common chickweed control 4 WAT was 78% by either dose of thaxtomin A applied POST, equivalent to that of the standard auxinic herbicide. However, 2013

Table 5. POST control of container-grown henbit, marsh yellowcress, flexuous bittercress, dandelion, and common chickweed using bioherbicides 42 d after two applications. Research conducted at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

Herbicide	Rate	Units ^a	Henbit		Marsh yellowcress		Flexuous bittercress		Dandelion		Common chickweed	
			2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
			% control ^b									
<i>Phoma macrostoma</i>	3,250	mu m ⁻²	0	5	16	18	2	10	10	3	2	2
<i>P. macrostoma</i>	6,500	mu m ⁻²	0	5	42	10	16	14	20	7	0	5
<i>P. macrostoma</i>	13,000	mu m ⁻²	0	10	54	45	14	18	28	22	0	5
Thaxtomin A	190	g ai ha ⁻¹	56	63	26	66	28	76	0	22	2	28
Thaxtomin A	380	g ai ha ⁻¹	98	95	74	95	62	100	8	77	20	65
Weed B Gon ^c	1,250	g ae ha ⁻¹	24	82	100	100	100	100	85	100	98	100
LSD _{0.05}			11	15	24	26	15	19	34	15	7	11

^a Abbreviation: mu, macrocidin units.

^b Percent control based on visual estimations of reductions in aboveground plant biomass compared with nontreated plots, where 0 = no reduction and 100 = complete plant mortality.

^c Ready-to-use formulation of auxinic herbicides containing 0.026% dicamba, 0.605% 2,4-dichlorophenoxyacetic acid, and 0.149% methylchlorophenoxypropionic acid.

Table 6. POST control of common chickweed, sparrow vetch, henbit, dandelion, and overall broadleaf weeds in newly seeded tall fescue using bioherbicides 28 d after application. Research conducted at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

Herbicide	Rate	Units ^a	Common chickweed		Sparrow vetch		Hop clover		Henbit ^b	Dandelion	Combined broadleaf weeds ^c	
			2012	2013	2012	2013	2012	2013			4 WAT	28 WAT
-----% control ^d -----												
<i>Phoma</i>												
<i>macrostoma</i>	6,500	mu m ⁻²	5	10	28	53	23	15	24	40	25	29
<i>P. macrostoma</i>	13,000	mu m ⁻²	10	10	20	58	45	20	25	60	32	44
Thaxtomin A	190	g ai ha ⁻¹	78	13	20	23	58	5	50	34	36	25
Thaxtomin A	380	g ai ha ⁻¹	78	35	18	23	73	8	60	54	45	13
Weed B Gon ^e	1,250	g ae ha ⁻¹	85	93	90	80	90	80	32	50	70	91
LSD _{0.05}			9	14	28	13	28	10	14	21	11	20

^a Abbreviations: mu, macrocidin units; WAT, weeks after treatment.

^b Data for henbit, dandelion, and broadleaf weed control were pooled across years, as treatment-by-year effect was not significant.

^c Combined broadleaf weeds is an estimate of percent weed control for all broadleaf weeds in the treated area and includes those evaluated individually and any other dicot weeds present.

^d Percent control based on visual estimations of reductions in aboveground plant biomass compared with nontreated plots, where 0 = no reduction and 100 = complete plant mortality.

^e Ready-to-use formulation of auxinic herbicides containing 0.026% dicamba, 0.605% 2,4-dichlorophenoxyacetic acid, and 0.149% methylchlorophenoxypropionic acid.

chickweed control with thaxtomin A was $\leq 35\%$. Sparrow vetch was not controlled by thaxtomin A. *Phoma macrostoma* controlled sparrow vetch 53 to 58% in 2013 but $< 30\%$ in 2012. (Table 6). Thaxtomin A provided 50 to 60% control of henbit 4 WAT, which was better than the auxinic herbicide or *P. macrostoma* (Table 6). Dandelion control 4 WAT was 60 and 54% with 13,000 mu m⁻² *P. macrostoma* and 380 g ai ha⁻¹ thaxtomin A, respectively, and not different from the auxinic herbicide. Overall broadleaf weed control 4 WAT was $\leq 45\%$ for bioherbicide treatments, less than the 70% control observed with the auxinic herbicide (Table 6). The following spring, 28 WAT, broadleaf weed control in the auxinic herbicide plots had improved to 91%; weed control with *P. macrostoma* and thaxtomin A plots had not improved. These results suggest that weeds not controlled by *P. macrostoma* or thaxtomin A grew to fill the voids left when susceptible species were suppressed.

Enhancing Thaxtomin A Activity on Poorly Controlled Species. *POST Control of Henbit with Increased Dose.* Year and year-by-dose interactions were significant for the 17-WAT evaluations (Table 7); therefore, data for each year are presented

separately. Percent cover estimates and visual control evaluations were negatively correlated ($R < -0.90$); therefore, only data from visual evaluations are presented. Within the range of doses tested, a linear dose response was observed for henbit control with thaxtomin A (Table 7). Henbit control 5 WAT with 190, 380, and 570 g ha⁻¹ thaxtomin A was 50, 73, and 88% in 2013 and 25, 48, and 73% in 2014, respectively (Table 7). By 11 WAT no dose provided greater than 80% control, and control declined further by 17 WAT. Incomplete control of henbit suggests that additional measures besides increased dose will be required to achieve consistent and acceptable control.

PRE Followed by Early POST Control of Henbit. Treatment-by-year interactions were not significant; therefore, data were pooled across years for presentation. Henbit control with thaxtomin A 4 wk after PRE applications was 26 and 65% at 190 and 380 g ha⁻¹, respectively (Table 8). In contrast, pendimethalin provided 93% control. These results were similar to results in the PRE experiments reported above. After an early-POST second application, henbit control with thaxtomin A was 52% at 190 g ha⁻¹ and 91% at 380 g ha⁻¹, equal to pendimethalin (Table 8). Sixteen weeks after the

Table 7. POST control of henbit using thaxtomin A. Research conducted at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

Herbicide	Rate g ai ha ⁻¹	Henbit control ^a		
		5 WAT ^b	11 WAT	17 WAT
		%		
2013 Experiment				
Thaxtomin A	190	50	25	5
Thaxtomin A	380	73	56	40
Thaxtomin A	570	88	79	65
<i>R</i> ² for linear dose response		0.79	0.86	0.85
P value		< 0.0001	< 0.0001	< 0.0001
2014 Experiment				
Thaxtomin A	190	25	43	38
Thaxtomin A	380	48	75	53
Thaxtomin A	570	74	80	73
<i>R</i> ² for linear dose response		0.848	0.726	0.744
P value		< 0.0001	< 0.0004	< 0.0003
ANOVA ^c of main effects and interaction				
Dose		< 0.0001	< 0.0001	< 0.001
Year		< 0.0046	0.024	0.0022
Dose by year		NS	NS	0.035

^a Percent control based on visual estimations of reductions in aboveground plant biomass compared with nontreated plots, where 0 = no reduction and 100 = complete plant mortality.

^b Abbreviations WAT, weeks after treatment; NS, not significant.

^c Probability values for significant *F*-tests from ANOVA for main effects and thaxtomin A dose-by-year interaction.

second application of thaxtomin A at 380 g ha⁻¹, henbit control was 86% and still equivalent to pendimethalin.

The results of these experiments demonstrate that both *P. macrostoma* and thaxtomin A have potential to provide commercially acceptable control of certain broadleaf weed species in turfgrass. However, results in field and container tests were inconsistent, and both products lacked efficacy on important weed species. *Phoma macrostoma* provid-

ed effective PRE and POST control of seedling dandelion in container and field tests but was less effective on other weed species tested. Similarly, Bailey et al. (2011) reported 92% mortality of dandelion, and poor control of henbit. However, in contrast to our results, Bailey et al. (2011) reported 68 to 96% mortality of common chickweed and 79% mortality of yellow woodsorrel after applications of *P. macrostoma*. These differences may be related to differences in product formulation and

Table 8. Control of henbit using PRE followed by an early-POST application of thaxtomin A. Research conducted at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

Herbicide	Rate g ai ha ⁻¹	Henbit control ^a		
		4 WAT ^b	4 WAT2	16 WAT2
		%		
Thaxtomin A	190	26	52	39
Thaxtomin A	380	65	91	86
Pendimethalin	3300	93	95	92
LSD _{0.05}		15	8	10

^a Percent control based on visual estimations of reductions in aboveground plant biomass compared with nontreated plots, where 0 = no reduction and 100 = no weeds present. Data were pooled across years, as treatment-by-year interaction was not significant.

^b Abbreviations: WAT, weeks after treatment; WAT2, weeks after second treatment.

application rates, as the rate of *P. macrostoma* was not reported in those studies. Plant growth stage and environmental conditions during and after application may have also played a part, as past attempts to utilize live pathogens as bioherbicides have demonstrated (Harding and Raizada 2015).

PRE applications of *P. macrostoma* in newly seeded turfgrass suppressed broadleaf weeds by 60% for 10 wk (Table 3) with no injury to tall fescue seedlings. Similarly, Bailey et al. (2009) reported no turfgrass injury when *P. macrostoma* was applied at seeding. Thus *P. macrostoma* may have utility for suppression of certain broadleaf weeds in newly seeded turf. However, *P. macrostoma* lacked efficacy on several winter annual broadleaf weed species, including common chickweed, which is one of the 10 most common weeds of southern turfgrass (Webster 2012).

Thaxtomin A at 380 g ha⁻¹ applied PRE or POST in newly seeded tall fescue suppressed henbit, dandelion, and field madder 50 to 70% but did not control common chickweed. Control on other species was variable. PRE followed by an early-POST application of thaxtomin A at 380 g ha⁻¹ resulted in improved henbit control and should be evaluated on other broadleaf weed species. Thaxtomin A applied at seeding injured tall fescue, but no turfgrass injury was observed when applied 7 to 8 wk after seeding. Additional research is needed to determine seedling turfgrass tolerance to thaxtomin A. Both thaxtomin A and *P. macrostoma* lacked efficacy on certain broadleaf weeds common in southern turfgrass and provided inconsistent results in these tests. Additional research is needed to improve consistency under field conditions and to expand the spectrum of weed species controlled.

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