

Final Report

EVALUATIONS OF BIOLOGICALLY BASED ALTERNATIVES FOR BROADLEAF WEED CONTROL IN TURF AND ORNAMENTALS

Joseph C. Neal
Department of Horticultural Science
North Carolina State University
Raleigh NC 27695
joe_neal@ncsu.edu

Introduction

Turfgrass and landscape management, and nursery crop production are multi-billion dollar industries in the United States. Traditional pest management programs in these sites rely heavily upon synthetic chemistry for insect, disease and weed management. However, significant concerns exist over exposure to lawn care and landscape maintenance chemicals applied in urban watersheds. Interest in alternative and organic pest management options for urban lawns and landscapes continues to grow, with retail sales of organic lawn and garden products estimated to be \$460 million in 2008, a gain of 12% over 2007. Yet few bio-based alternatives for weed control are available. Non-selective weed control products such as vinegar and pelargonic acid are available to the market. However, aside from hand weeding, traditional synthetic herbicides are the only effective options for selective weed control in turfgrass, landscapes or nursery crops. Fe-HEDTA is currently labeled for control of certain broadleaf weeds in turf, but research on its efficacy has been lacking.

Recently, registration packages for two bio-based products for selective broadleaf weed control in turf have been submitted to US-EPA, *P. macrostoma* and MBI-005. *Phoma macrostoma* is a fungus isolated from Canada thistle (*Cirsium arvense*). Experiments conducted in Canada have demonstrated efficacy on common broadleaf weeds of turfgrass and landscape plantings (Bailey et al. 2009) with limited mobility and persistence in soil (Bailey and Derby 2005; Zhou et al. 2004). The active ingredient in MBI-005 is thaxtomin, a non-systemic plant toxin produced by *Streptomyces acidiscabies RL-110* during fermentation. Thaxtomin phytotoxins have been characterized and shown to have herbicidal properties with species selectivity varying with changes in molecular structure (King and Lawrence 2001).

From 2010 to 2013 we conducted field, greenhouse and growth chamber research on these three bio-based products. Results from these trials have demonstrated that *P. macrostoma* and MBI-005 control many common broadleaf weed species when applied preemergence or postemergence. However, poor or variable control of some weed species was observed. For example: MBI-005 lacks efficacy on white clover, one of the most common broadleaf weeds of cool-season turf. *Phoma macrostoma* provided nearly complete control of white clover at low doses but lacked efficacy on henbit. Fe-HEDTA was ineffective on common chickweed.

Additional research is needed to clearly define the spectrum of weeds controlled with each product and strategies to broaden the spectrum of control.

The experiments reported herein explore in more detail the spectrum of weeds controlled by preemergence and postemergence applications of these biopesticides, application strategies to optimize efficacy on target weeds, and safety of biopesticides to established and seeded cool-season turf. Additionally, weed control products used in landscape settings must not pose a risk of injury to desirable ornamental plants. Experiments were conducted to determine the potential for injury to ornamental plants as well as potential uses for biopesticides for weed control in ornamental plant production. Specifically tests were conducted to evaluate:

- Preemergence and postemergence efficacy of Thaxtomin A and Phoma macrostoma on common dicot and grassey weeds;
- Application strategies for improving control of difficult to control weeds – henbit and chickweed;
- Dose and application frequencies for residual control of summer annual and winter annual grassey weeds in turf;
- Safety of biopesticide applications to established and seedling turfgrasses;
- Optimizing Fe-HEDTA dose, spray volume and concentrations for broadleaf weed control in turf; and
- Potential for injury to landscape ornamentals from biopesticide applications.

The results are organized into five parts.

<u>Section #</u>	<u>Topic</u>	<u>Page #</u>
Part I	Selective Broadleaf Weed Control in Turf With the Bioherbicides <i>Phoma macrostoma</i> , Thaxtomin A (MBI-005)	3
Part II	Efficacy of the Bioherbicide Thaxtomin A on Smooth Crabgrass and Annual Bluegrass, and Safety in Cool-Season Turfgrasses	23
Part III	Postemergence Broadleaf Weed Control with the Bioherbicide FeHEDTA	45
Part IV	Safety of biopesticide applications to ornamental plants	70
Part V	Bio-based product comparison for the control of dicondra in turf	74

Part I

Selective Broadleaf Weed Control in Turf With the Bioherbicides *Phoma macrostoma*, Thaxtomin A (MBI-005)

Abstract

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 at least one of two years of container experiments, PRE applications of *P. macrostoma* controlled dandelion, marsh yellowcress and flexuous bittercress as well as pendimethalin but did not control yellow woodsorrel, henbit, hairy galinsoga, common chickweed, or annual bluegrass as well as pendimethalin. 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 at 64% and 72% four weeks after treatment and declined afterwards, suggesting that these bioherbicides possess short residuals and therefore must be re-applied for season-long control. Overall POST broadleaf weed control using *P. macrostoma* and thaxtomin A was only 41% and 25%, respectively. In separate experiments, higher doses of thaxtomin A improved POST control of henbit, which was not well-controlled in other experiments, and PRE followed by early POST applications of thaxtomin A provided $\geq 86\%$ henbit control, equal to pendimethalin. These results suggest that both *P. macrostoma* and thaxtomin A are capable of controlling certain broadleaf weeds in turfgrass, but both lack efficacy on some important weed species, particularly chickweed. Thaxtomin A efficacy on henbit was improved with increasing dose and by PRE followed by early POST applications.

Nomenclature: *Phoma macrostoma*; thaxtomin A; 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; flexuous bittercress, *Cardamine flexuosa* With., CARFL; henbit, *Lamium amplexicaule* L., LAMAM; common chickweed, *Stellaria media* (L.) Vill., STEME; tall fescue, *Lolium arundinaceum* (Schreb.) S.J. Darbyshire ‘The Rebels’, ‘Top Choice’, FESAR; sparrow vetch, *Vicia tetrasperma* (L.) Schreb., VICTE; field madder, *Sherardia arvensis* L., SHRAR.

Key words: biocontrol, biological weed control; biopesticide; macrocidin.

Introduction

In the United States, turfgrass occupies more than 30 million acres of land, including some 50 million lawns plus golf courses, athletic fields, parks, and sod farms (Grewal 1999). In total, managed turfgrass area represent roughly 23% of developed land in the United States (Hernke and Podein 2011). Turf managers aim to maintain healthy, homogenous and weed-free turfgrass in order 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. Between 2000 and 2007, Grube et al. (2011) estimated that roughly 45 million kg of synthetic herbicides were applied to non-crop areas in the United States. In 1996 over \$200 million was spent on synthetic herbicides for use in highly maintained turf in the United States, with sales predicted to increase 2-4% 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, such as the state of New York's Child Safe Playing Field Act, which banned the use of synthetic chemical pesticides on school properties (Grant 2011). These restrictions have heightened the need for alternative weed management strategies in turfgrass.

Biological weed control is one alternative to synthetic herbicides which has received considerable attention in recent years, though results have been mixed (Boyetchko et al. 2009; Chandler et al. 2011; Ghosheh 2005). While the global market for biopesticides has grown over the past decade, with projected sales over \$1 billion in 2010, bioherbicides make up only a small portion of the total biopesticide market (Bailey et al. 2010; Thakore 2006). Developing successful bioherbicides has proven challenging, as past bioherbicides have frequently offered a narrow spectrum of control and their effectiveness could be significantly affected by environmental conditions at the time of application, limiting their commercial potential (Chandler et al. 2011; Johnson 1994).

There are currently few biologically-based weed control options available to turf 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 (Patton and Weisenberger 2011; St. John and DeMuro 2013; Siva 2014). 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 turf. FeHEDTA, a chelated iron formulation, was registered by the United States 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). While FeHEDTA has been shown to effectively control broadleaf weeds in turf, and is classified by

the US EPA as a biopesticide, but due to the synthetic chelating agent it is not an option in certified organic systems. *Sclerotinia minor*, a plant pathogen which has been extensively researched as a potential biological control agent for broadleaf weeds in turf, 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 which have struggled to achieve commercial success, the efficacy of *Sclerotinia minor* can vary greatly depending on environmental factors such as moisture and temperature, which presents an obstacle for turf 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 lack options.

Two other biologically-based products which have been reported to have potential for the control of broadleaf weeds in turfgrass are *P. 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. Our preliminary data indicate that the pathogen remains infectious even at higher temperatures common in the Southern U.S., suggesting that warmer environmental conditions would not limit the efficacy of *P. macrostoma* (Neal et al. 2013). However, no data are currently available on *P. macrostoma* efficacy in warmer climates such as the southern United States.

Thaxtomin A is a chemical produced by the bacterium *Streptomyces scabies* which 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 1992). Existing data characterizing the herbicidal activity of thaxtomin A is scarce. King and Lawrence (2001) demonstrated that thaxtomin A is non-systemic 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 when applied POST, though symptoms were observed at both application timings. While these initial screenings identified thaxtomin A as possessing 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 prior to commercialization.

While both *P. macrostoma* and thaxtomin A have shown promise 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 which 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 compared conventional and bioherbicide standard products, 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 (35.79N, -78.7W). 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 pre-weighed aliquots using a hand-held 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 Road, Wheaton, IL 60187) calibrated to deliver 280 L ha⁻¹. Weed control was visually evaluated on a 0 to 10 scale where 0 = no control (weed populations and cover were equal to the non-treated areas) and 10 = 100% control (no living weed biomass present). Intermediate values are visual estimates of percent reduction in above ground plant biomass compared to the non-treated, abbreviated to a 0 to 10 scale. These ratings were converted into percent control for presentation. 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.* 1 L pots were filled with a hammer-milled pine bark nursery substrate (Parker Bark Company, 3295 US 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 at a rate of approximately 100 seeds per pot on September 13, 2012 and the test was repeated on September 16, 2013. Weed species were: yellow woodsorrel (*Oxalis stricta*), henbit (*Lamium amplexicaule*) marsh yellowcress (*Rorippa palustris*), flexuous bittercress (*Cardamine flexuosa*), hairy galinsoga (*Galinsoga quadriradiata*), dandelion (*Taraxacum officinale*), common chickweed (*Stellaria media*), ivyleaf speedwell (*Veronica hederifolia*) and annual bluegrass (*Poa annua*). Seeds of all weed species were locally collected in previous years. After seeding, pots were overhead irrigated to settle the seeds and substrate.

Treatments were applied immediately after seeding, and included a non-treated control, thaxtomin A at 190 and 380 g ai ha⁻¹, and three doses of *P. macrostoma*. *P. macrostoma* used in these experiments was applied on granules containing approximately 400 macrocidin units (mu's, a proprietary standardized measure of macrocidin content) g⁻¹ at rates of 3250, 6500, and 13000 mu m⁻² (Bailey 2011). Two industry standard preemergence herbicide treatments were also included: corn gluten meal (Preen® Organic Weed Preventer, Lebanon Seaboard Corporation, 1600 East Cumberland Street, Lebanon, PA 17042) at 97 kg ha⁻¹, and pendimethalin (Pendulum®, BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709) at 3.3 kg ai ha⁻¹. After applications were made, pots were maintained outdoors and received 0.5 cm of overhead irrigation three times per day.

The experiments were arranged in a randomized complete block design with 5 replications in 2012 and 6 replications in 2013 (a single pot of each weed species was included per treatment per replicate). Beginning one week after the initial application, weed control was visually evaluated weekly on the 0 to 10 scale described above.

PRE Weed Control in Seedling Tall Fescue Turf. 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 two weeks before seeding. Prior to seeding, debris was removed and the sites were raked smooth, core cultivated, and amended with a 9-13-7 (N-P₂O₅-K₂O) fertilizer (Ferti-lome New Lawn Starter Fertilizer®, Voluntary Purchasing Group, 230 FM 87, Bonham, Texas 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 Road NE, Salem, OR 97305). In both years, turf 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 four weeks after seeding. Following establishment, trial areas were mowed as needed at a 7.6 cm height with a rotary mower, with clippings returned to the site, and the areas received supplemental irrigation as-needed to prevent drought stress.

Initial PRE treatments were applied on September 27, 2012 and September 9, 2013, with re-treatment occurring on November 16, 2012 and October 9, 2013. PRE treatments included thaxtomin A at 190 and 380 g ai ha⁻¹, *P. macrostoma* at 6500 and 13000 mu m⁻², corn gluten meal at 97 kg ha⁻¹, and a non-treated control. In 2012, spray treatments were applied using a CO₂ pressurized backpack sprayer equipped with two 8008 flat fan nozzles calibrated to deliver 467 L ha⁻¹. In 2013, spray treatments were applied using the same CO₂-pressurized backpack sprayer equipped with two 8006 flat fan nozzles calibrated to deliver 280 L ha⁻¹.

Evaluations were conducted weekly for eight weeks, after which low temperatures inhibited further weed germination. A final evaluation was conducted the following spring, approximately 32 weeks after initial applications, to evaluate the long-term impact of PRE treatments on weed populations. Control of each weed species present was evaluated visually on the 0-10 scale described above. Four weeks after treatment, individual weed counts were also conducted for each species uniformly distributed in the field.

POST Weed Control. POST Weed Control in Containers. Methods used in these experiments were identical to those used in the PRE weed control container experiment described above, except for the following. In the POST efficacy experiment treatments were applied 4 weeks after seeding on October 16, 2012 and October 18, 2013 when weeds had reached an average height of 2 to 5 cm, and re-applied 3 weeks later on November 6 in both 2012 and 2013. Treatments in the POST experiment included a non-treated control, thaxtomin A at 190 and 380 g ai ha⁻¹, *P. macrostoma* at 3250, 6500, and 13000 mu m⁻², and a ready-to-use auxin herbicide spray (Weed B Gon MAX RTU®, 0.026% dicamba, 0.605% 2,4-dichlorophenoxyacetic acid, 0.149% methylchlorophenoxypropionic acid; The ORTHO Group, 1411 Scottslawn Road, Marysville, OH 43040) at approximately 412 g ai ha⁻¹. Pots

used in the POST experiments were maintained in a covered shade house after treatments were applied.

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 on November 16, 2012 (7 weeks after seeding) and November 4, 2013 (8 weeks after seeding). Treatments included a non-treated control, thaxtomin A at 190 and 380 g ai ha⁻¹, *P. macrostoma* at 6500 and 13000 mu m⁻², and Weed B Gon MAX RTU at 412 g ai ha⁻¹. At the time POST treatments were applied, dandelion, common chickweed, henbit, and sparrow vetch (*Vicia tetrasperma*) were the most common weed species present. Weed control was visually evaluated 1, 2, and 4 weeks after application, after which cold temperatures had rendered plants dormant.

Enhancing Thaxtomin A Activity On Poorly Controlled Species. Two strategies for enhancing thaxtomin A efficacy on poorly controlled species were compared. Henbit, which was susceptible but not consistently well-controlled PRE or POST by thaxtomin A in prior studies, was chosen as a model species to evaluate these strategies. 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 and 570 g ai ha⁻¹, as well as a non-treated control. Field beds were cultivated in early September to stimulate winter annual weed germination. Treatments were applied on October 25, 2013 and the experiment was repeated on October 24, 2014. At the time of application a natural population of henbit was present in all plots, averaging 70-80% cover and growth stages from cotyledon to four leaf pairs with an average height of 7.5cm. Other weeds present in lower populations included shepherd's-purse (*Capsella bursa-pastoris*) and common chickweed. Percent control of each weed species present at the time of treatment was evaluated weekly for 16 weeks. The 0-10 scale described above. Visual estimations of percent weed cover were also recorded at each evaluation.

PRE Followed-By Early POST Control of Henbit. Treatments in this experiment included thaxtomin A at 190 and 380 g ai ha⁻¹, pendimethalin at the labelled rate of 3.3 kg ai ha⁻¹, and a non-treated control. In both years, field plots were established in a recently-tilled planting bed with a history of heavy henbit and common chickweed infestation. PRE treatments were applied on September 20, 2013 on October 2, 2014. Early POST treatments were re-applied four weeks 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 non-treated plots, while henbit was present at low populations in treated plots and varied from cotyledons to two leaf pairs. Evaluations were initiated four weeks after the initial application, after a sufficient population of henbit had emerged. Thereafter evaluations took place weekly. Evaluation methods were identical to those described in the experiment evaluating POST control of henbit above.

Statistical Analysis. Data collected from all experiments were subjected to analysis of variance using Proc GLM in SAS (SAS 9.4, SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC 27513) to test for both main treatment effects and interactions. Where treatment effects were determined to be significant, treatment means were separated using Fisher's

protected least significant difference 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. Both *P. macrostoma* and thaxtomin A controlled some weed species equivalent to pendimethalin. For both *P. macrostoma* and thaxtomin A, percent weed control increased with increasing dose. Treatment-by-year interactions were significant for all species, thus data are presented by year.

In 2012, *P. macrostoma* at 3250 and 6500 μm^{-2} provided $\geq 90\%$ control of marsh yellowcress and dandelion, equal to pendimethalin, but provided $\leq 58\%$ control of all other species (Tables 1 & 2). At 13000 μm^{-2} , *P. macrostoma* provided $\geq 95\%$ control of marsh yellowcress, flexuous bittercress, and dandelion, all equal to the pendimethalin treatment, but $\leq 63\%$ control of all other species (Tables 1 & 2).

Weed control with *P. macrostoma* was lower in 2013 than in 2012. In 2013, *P. macrostoma* at 3250 and 6500 μm^{-2} provided 32% and 58% control of dandelion, respectively, which was equal to pendimethalin, but provided $\leq 53\%$ control of all other species (Tables 1 & 2). At 13000 μm^{-2} , *P. macrostoma* controlled dandelion 100%. Marsh yellowcress, flexuous bittercress, and hairy galinsoga were controlled 87%, 65%, and 32%, respectively. All other species were controlled $\leq 27\%$ (Tables 1 & 2).

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, but control of all other species was $\leq 78\%$ and was not equal to pendimethalin (Tables 1 & 2). Thaxtomin A at 380 g ai ha^{-1} provided $\geq 93\%$ control of yellow woodsorrel, henbit, marsh yellowcress, dandelion, and annual bluegrass, all equivalent to pendimethalin (Tables 1 & 2). Thaxtomin A also provided 75% control of hairy galinsoga, equal to pendimethalin (Table 2). Thaxtomin A at 380 g ai ha^{-1} also provided 60% control of flexuous bittercress and 20% control of common chickweed, neither of which were equivalent to pendimethalin (Tables 1 & 2).

PRE weed control with thaxtomin A was consistently lower in 2013 than in 2012. Thaxtomin A at 190 g ai ha^{-1} provided $\leq 45\%$ control of all species tested in 2013. Only hairy galinsoga and dandelion were controlled equal to pendimethalin, which provided only 40 and 42% control, respectively. At 380 g ai ha^{-1} , thaxtomin A provided $\leq 60\%$ control of all species in 2013.

The reasons for the drastic decrease in thaxtomin A efficacy from 2012 to 2013 are unclear. Product samples from each year were compared in a bioassay with no differences observed (data not shown). Furthermore, POST efficacy was similar between years; thus bioactivity of the product sample was dismissed 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. In 2012, the site received 7.9 cm of rainfall in the week after treatments were applied, and received 3.5 cm in 2013; the differences in rainfall between years may be significant, as Hoskins et al. (2014) demonstrated that solute transport through

pine bark substrates is more rapid under unsaturated conditions. Differences in substrate between 2012 and 2013 may have also played a part; while both experiments were conducted in a 100% pine bark substrate, such substrates can differ greatly in their textural and physical properties. It is possible that these textural and compositional differences may have affected the residual of the bioherbicides in question, as 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). Substrate pH also varied between years, 5.6 in 2012 and 3.8 in 2013. Differences in soil pH have been shown to impact the persistence and phytotoxicity of certain herbicides (Grey & McCullough 2012; Rosenkrantz et al. 2014). However, no differences in thaxtomin A PRE efficacy were observed in a bioassay comparing pine bark substrates with pH adjusted to 4.0 and 5.8 (data not shown). Thus, further exploration into the influence of environmental factors on the efficacy and persistence of thaxtomin A is necessary.

Symptoms observed on weeds treated with *P. macrostoma* were consistent with those reported by Bailey et al. (2009), and included bleaching, chlorosis, and in some cases eventual mortality. However several species, including yellow woodsorrel, henbit, and hairy galinsoga, displayed chlorosis shortly after emergence but eventually recovered. Injury symptoms on plants treated with thaxtomin A included seedling stunting and warping of the growing points, common symptoms of cellulose biosynthesis inhibition as described by King and Lawrence (2001). As with *P. macrostoma*, however, several weed species eventually recovered from this injury suggesting that increased dosing or additional treatments might be required to obtain commercially acceptable weed control.

PRE Weed Control in Seedling Tall Fescue Turf. Percent control ratings were negatively correlated with individual weed counts ($R = -0.77$ and -0.81 in 2012 and 2013, respectively), thus only percent control data is presented. Treatment-by-year interactions were non-significant for all species; therefore, data were pooled across years for presentation. In general, control of most species increased as *P. macrostoma* and thaxtomin A doses increased. Injury symptoms caused by *P. macrostoma* and thaxtomin A were consistent with those observed in containers, as described above.

Corn gluten meal did not control henbit, dandelion, chickweed or field madder (Table 3). When evaluated 4WAT, corn gluten provided no control of broadleaf weeds. Control improved at subsequent evaluations, providing 20% control at 10WAT and 39% control at 32WAT.

Phoma macrostoma at 6500 mu m⁻² controlled dandelion 87%, but all other species were controlled $\leq 19\%$ (Table 3). At 13000 mu m⁻², *P. macrostoma* provided 64% control of henbit and 99% control of dandelion, but provided $\leq 28\%$ control of common chickweed and field madder (Table 3). When evaluated 4WAT and 10WAT, overall broadleaf weeds were controlled 24 to 27% at 6500 mu m⁻² compared to 61 to 64% at 13000 mu m⁻², respectively. When evaluated 32WAT, overall broadleaf weed control was 42-50%, with no differences between doses of *P. macrostoma* (Table 3).

Thaxtomin A at 190 g ai ha⁻¹ controlled henbit 51% and field madder 41%, but provided $\leq 21\%$ control of dandelion and common chickweed (Table 3). Overall broadleaf weed control with thaxtomin A at 190 g ai ha⁻¹ was 54% when evaluated 4WAT, but declined to 47% at 10

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

Tall fescue establishment was not affected by *P. macrostoma* treatments, but was significantly hindered by both rates of thaxtomin A. Ten weeks after treatment, turf cover in the non-treated plots was 72% whereas turf 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 while thaxtomin A does have the potential to control broadleaf weeds when applied preemergence, it will also significantly reduce tall fescue cover. 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, thus 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; thus, only data from the final evaluation, six weeks after the second treatment, are presented here.

The industry standard herbicide, Weed B Gon RTU, provided similar control of most species in 2012 and 2013 (79-100%), with the exception of 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, and no *P. macrostoma* treatment controlled any species in the experiment equal to the auxinic herbicide (Tables 4 and 5).

At 190 g ai ha⁻¹, thaxtomin A provided 82% control of ivyleaf speedwell, equal to the synthetic auxin mixture. This rate also provided 79% control of yellow woodsorrel and 60% control of hairy galinsoga, which was greater than that provided by the auxinic herbicide (Table 4). In the 2012 experiment, thaxtomin A at 190 g ai ha⁻¹ provided 56% control of henbit, which was equivalent to the auxinic herbicide, but provided $\leq 28\%$ control of marsh yellowcress, flexuous bittercress, dandelion, and common chickweed, none of which were equal to the auxinic herbicide (Table 5). In the 2013 experiment, control of henbit, marsh yellowcress, and flexuous bittercress with thaxtomin A at 190 g ai ha⁻¹ was $\geq 63\%$, but was not equal to the auxinic herbicide. Dandelion and common chickweed were controlled 22% and 28%, respectively (Table 4).

In both years, thaxtomin A at 380 g ai ha⁻¹ provided $\geq 80\%$ control of yellow woodsorrel, hairy galinsoga and ivyleaf speedwell, equal to or better than the auxinic herbicide (Table 4). Thaxtomin A at 380 g ai ha⁻¹ controlled henbit 95% to 98%, and was better than the auxinic herbicide in 2012. Marsh yellowcress, flexuous bittercress, dandelion, and common chickweed were all controlled $\leq 74\%$ in 2012, not equal to the auxinic herbicide (Table 5). Control with thaxtomin A at 380 g ai ha⁻¹ was improved in 2013, providing $\geq 95\%$ control of henbit, marsh yellowcress, and flexuous bittercress, equal to the auxinic herbicide. Control of dandelion and chickweed also improved to 77% and 65%, respectively, but was not equal to

the auxinic herbicide (Table 5).

Plants typically displayed some chlorosis or bleaching following applications of *P. macrostoma*, but recovery was rapid following application. Complete bleaching and necrosis were not observed on any species after applications of *P. macrostoma*. Injury caused by thaxtomin A typically presented with stunting and warped growth, followed by wilting and eventual necrosis of some species. This is consistent with the observations of King and Lawrence (2001).

Bailey et al. (2011) reported that henbit was not well controlled by *P. macrostoma*, which these data confirm. The rest of these results differed significantly from those reported by Bailey et al. (2011), who reported 92% mortality of dandelion, 68-96% mortality of common chickweed, and 79% mortality of yellow woodsorrel following applications of *P. macrostoma*. These differences may be related to differences in product formulation and application rates, as the rate of *P. macrostoma* was not reported in these 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 (Johnson 1994).

POST Weed Control in Seedling Tall Fescue Turf. Treatment-by-year interactions were significant for common chickweed and sparrow vetch, thus data for these species are presented by year. For all other species treatment by year interactions were not significant and data were pooled across years.

Two applications of *P. macrostoma* provided $\leq 10\%$ control of common chickweed in both years when applied POST (Table 6). This is consistent with results in the container experiment. In 2012, common chickweed was controlled 78% by both rates of thaxtomin A applied POST, equivalent to that of the standard auxinic herbicide mixture. However, in 2013 chickweed control with thaxtomin A was $\leq 35\%$. Variable chickweed control was also observed in the container experiment.

Neither thaxtomin A nor *P. macrostoma* controlled sparrow vetch equal to the auxinic herbicide (Table 6). Thaxtomin A applied at 190 and 380 g ai ha⁻¹ provided 50 and 60% control of henbit, respectively, which was better than the auxinic herbicide. *P. macrostoma* provided about 25% control of henbit, equal to the auxinic herbicide (Table 6). All bioherbicide treatments provided $\geq 34\%$ control of dandelion, equal to that of the auxinic herbicide. However, when evaluated for total overall broadleaf weed control, all bioherbicide treatments provided $\leq 41\%$ control, and none were equal to the auxinic herbicide (Table 6). These results suggest that weeds not controlled by the two biocontrol agents grew rapidly to fill the voids left when other species were controlled.

Enhancing Thaxtomin A Activity on Poorly Controlled Species. Treatment-by-year interactions were non-significant for henbit control in these experiments, thus data were pooled across years for presentation.

POST Control of Henbit With Increased Dose. At all rating dates, henbit control improved as thaxtomin A dose increased. When evaluated 4 weeks after the application, thaxtomin A provided 37%, 60%, and 81% control of henbit when applied at 190, 380, and 570 g ai ha⁻¹, respectively (Table 7). Plant injury symptoms persisted until 10 weeks after treatment, when

the same rates of thaxtomin A provided, 34%, 66%, and 79% control of henbit. By 16 weeks after application, control of weeds had declined to 21%, 46%, and 69% for thaxtomin A applied at 190, 380, and 570 g ai ha⁻¹, respectively (Table 7). This incomplete control of henbit suggests that additional measures besides increased dose will be required to achieve consistent and acceptable henbit control.

PRE Followed by Early POST Control of Henbit. When evaluated four weeks after preemergence applications, henbit was controlled 26% and 65% by thaxtomin A at 190 and 380 g ai ha⁻¹, respectively (Table 8). In contrast, pendimethalin provided 93% control. These results were similar to results in the PRE experiments reported above. Following a second, early postemergence application, henbit was controlled 51% by thaxtomin A at 190 g ai ha⁻¹ and 91% by thaxtomin A at 380 g ai ha⁻¹, equal to pendimethalin (Table 8). Sixteen weeks after the second application of thaxtomin A at 380 g ai ha⁻¹, henbit control was 86%, also equivalent to pendimethalin.

The results of these experiments demonstrate that both *P. macrostoma* and thaxtomin A have the potential to provide commercially acceptable control of certain weed species when applied PRE, and can suppress several others. The results further suggest that PRE and POST applications of thaxtomin A can control of certain broadleaf weed species. PRE followed by early POST applications of thaxtomin A provided better control of henbit, which suggests that control programs utilizing thaxtomin A applied PRE followed by additional POST applications could provide significantly improved control. While neither bioherbicide controlled all species tested, these results suggest that they can provide control of several species comparable to an industry standard synthetic auxin herbicide under field conditions typically observed in turfgrass systems.

Literature Cited

Abu-Dieyeh MH, Watson AK (2007) Population dynamics of broadleaf weeds in turfgrass as influenced by chemical and biological control methods. *Weed Sci* 55:371–380

Anonymous (2009) Proposed Registration Action Document: Iron HEDTA. United States Environmental Protection Agency. http://www.epa.gov/pesticides/chem_search/reg_actions/registration/decision_PC-034702_17-Sep-09.pdf. Accessed: July 10, 2014. 16 p

Bailey KL, Boyetchko SM, Längle T (2010) Social and economic drivers shaping the future of biological control: A Canadian perspective on the factors affecting the development and use of microbial biopesticides. *Biol Control* 52(3):221-9

Bailey K, Falk S, Lombardo S (2009) Status of phoma macrostoma, a bioherbicide for broadleaved weed control in turfgrass. Pages 40-42 in *Proc. IXth International Bioherbicide Group Workshop*. Orlando, FL

Bailey KL, Pitt WM, Falk S, Derby J (2011) The effects of *Phoma macrostoma* on nontarget plant and target weed species. *Biol Control* 58(3):379-386

Bélair G, Koppenhöfer AM, Dionne J, Simard L (2010) Current and potential use of pathogens in the management of turfgrass insects as affected by new pesticide regulations in North America. *Int J Pest Manage* 56(1):51-60

- Bourdôt GW, Saville DJ, Jong MD (2011) Evaluating the environmental safety of broad-host-range bioherbicides. *Pest Technol* 5:34-40
- Boyetchko SM, Bailey KL, De Clerck-Floate RA (2009) Current biological weed control agents - their adoption and future prospects. *Prairie Soils and Crops* 2:6.
- Chandler D, Bailey AS, Tatchell GM, Davidson G, Greaves J, Grant WP (2011) The development, regulation and use of biopesticides for integrated pest management. *Philos Trans R. Soc, B* 366(1573):1987-98
- Elfort EMA, Tardif FJ, Robinson DE, Lyons EM (2008) Effect of perennial ryegrass overseeding on weed suppression and sward composition. *Weed Technol* 22:231-239
- Evans HC, Seier MK, Derby JA, Falk S, Bailey KL (2013) Tracing the origins of white tip disease of *Cirsium arvense* and its causal agent, *Phoma macrostoma*. *Weed Res* 53(1):42-52
- Fenoll J, Vela N, Navarro G, Perez-Lucas G, Navarro S (2014) Assessment of agro-industrial and composted organic wastes for reducing the potential leaching of triazine herbicide residues through the soil. *Sci Total Environ* 494:124-132.
- Fry BA, Loria R (2002) Thaxtomin A: evidence for a plant cell wall target. *Physiol Mol Plant Pathol* 60(1): 1-8
- Ghosheh HZ (2005) Constraints in implementing biological weed control: a review. *Weed Biol Manage* 5:83-92
- Grant J (2011). The child safe playing fields act: NY's ban on pesticide use on school and day care center grounds. http://www.hort.cornell.edu/turf/pdfs/school_ban_CUTT_2011.pdf. Accessed May 25, 2015
- Grewal PS (1999) Factors in the success and failure of microbial control in turfgrass. *Integrated Pest Manag Rev* 4(4):287-294
- Grey TL, McCullough PE (2012) Sulfonylurea herbicides' fate in soil: dissipation, mobility, and other processes. *Weed Technol* 26(3):579-581.
- Grube A, Donaldson D, Kiely T, Wu L (2011) Pesticide industry sales and usage: 2006 and 2007 estimates. Washington, DC: US Environmental Protection Agency. 41 p
- Hernke MT, Podein RI (2011) Sustainability, health and precautionary perspectives on lawn pesticides and alternatives. *EcoHealth*: 8:223-232
- Hoskins TC, Owen JS, Fields JS (2014) Solute transport through a pine bark-based substrate under saturated and unsaturated conditions. *J Am Soc Hortic Sci* 139(6):634-641
- King RR, Lawrence CH, Calhoun LA (1992) Chemistry of phytotoxins associated with *Streptomyces scabies*, the causal organism of potato common scab. *J Agric Food Chem* 40(5):834-837
- King RR, Lawrence CH, Gray JA (2001) Herbicidal properties of the thaxtomin group of phytotoxins. *J Agric Food Chem* 49(5):2298-2301
- Liu DLY, Christians NE, Garbutt JT (1994) Herbicidal activity of hydrolyzed corn gluten

meal on three grass species under controlled environments. *J Plant Growth Regul* 13(4):221-6

McDade MC, Christians NE (2000) Corn gluten meal--a natural preemergence herbicide: effect on vegetable seedling survival and weed cover. *Am J Alternative Agric* 15(4):189

Neal JC, Shew B, Schiavone R (2013) Temperature and dose influence *Phoma macrostoma* efficacy on broadleaf weeds. Pages 29-33 in Proc. IXth International Bioherbicide Group Workshop. Beijing, China

Patton A, Weisenberger D (2012) Evaluation of crabgrass control with various dimension formulations and corn gluten meal.

http://www.agry.purdue.edu/turf/report/2011/PDF/07_AGRY_Patton_crabgrass.pdf.

Accessed May 25, 2015

Porpiglia P, Towne O, Houseworth D (1996) Overview of the turf weed control market in the USA. *Pestic Sci* 47(4):387-388

Riddle G, Burpee L, Boland G (1991) Virulence of *Sclerotinia sclerotiorum* and *S. minor* on dandelion (*Taraxacum officinale*). *Weed Sci* 39:109-118

Rosenkrantz RT, Cedergreen N, Baun A, Kusk KO (2013) Influence of pH, light cycle, and temperature on ecotoxicity of four sulfonylurea herbicides towards *Lemna gibba*.

Ecotoxicology 22(1):33-41.

Schnick PJ, Stewart-Wade S, Boland GJ (2002) 2,4-D and sclerotinia minor to control common dandelion. *Weed Sci* 50(2):173-178

Siva C (2014) Alternative strategies for broadleaf weed management in residential lawns. MS thesis. Guelph, ON. The University of Guelph.

https://atrium.lib.uoguelph.ca/xmlui/bitstream/handle/10214/8046/Siva_Cynthia_201405_Msc.pdf?sequence=1. Accessed: May 24, 2015. 157 p

St. John R, DeMuro N (2013) Efficacy of corn gluten meal for common dandelion and smooth crabgrass control compared to nitrogen fertilizers. *Appl Turfgrass Sci*
DOI:10.1094/ATS-2013-0426-01-RS

Thakore Y (2006) The biopesticide market for global agricultural use. *Ind Biotechnol* 2(3):194-208

Wehtje G, Gilliam CH, Marble SC (2012) Duration of flumioxazin-based weed control in container-grown nursery crops. *Weed Technol* 26(4):679-683.

Wilen C (2012) Preliminary Report on Iron HEDTA: A Natural Selective Herbicide. Retail Nursery IPM Newsletter. <http://www.ipm.ucdavis.edu/PDF/PUBS/retailipmnews.2012.mar.pdf>. Accessed: July 10, 2014

Zimdahl R (2013) Fundamentals of weed science. 4th edn. San Diego, CA, USA: Elsevier. 664 p

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

Herbicide	Rate	Rate Unit ^a	Yellow woodsorrel		Henbit		Marsh yellowcress		Flexuous bittercress	
			2012	2013	2012	2013	2012	2013	2012	2013
-----% Control ^b -----										
<i>P. macrostoma</i>	3250	mu m ⁻²	10	15	3	17	90	37	36	27
<i>P. macrostoma</i>	6500	mu m ⁻²	8	15	20	7	100	53	58	25
<i>P. macrostoma</i>	13000	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	3300	g ai ha ⁻¹	100	100	100	98	98	98	83	85
LSD _{0.05}			24	27	25	31	12	36	19	26

^a Abbreviations: *P. macrostoma*, *Phoma macrostoma*; mu, macrocidin unit.

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

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

Herbicide	Rate	Rate Unit	Hairy galinsoga ^a		Dandelion		Common chickweed		Annual bluegrass	
			2012	2013	2012	2013	2012	2013	2012	2013
-----% Control ^b -----										
<i>P. macrostoma</i>	3250	mu m ⁻²	6	13	90	32	4	18	26	10
<i>P. macrostoma</i>	6500	mu m ⁻²	16	5	100	58	10	7	12	5
<i>P. macrostoma</i>	13000	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	3300	g ai ha ⁻¹	58	40	98	42	96	75	94	98
LSD _{0.05}			23	25	19	23	13	20	12	17

^a Abbreviations: *P. macrostoma*, *Phoma macrostoma*; mu, macrocidin unit.

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

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	Rate Unit	Combined Broadleaf							
			Weed control ^a , 4 WAT ^b				Weeds ^c			Tall
			Henbit	Dandelion	Common chickweed	Field madder	4 WAT	10 WAT	32 WAT	Fescue ^d , 10 WAT
-----% Control-----								-% Cover-		
<i>P. macrostoma</i>	6500	mu m ⁻²	19	87	13	12	27	24	50	72
<i>P. macrostoma</i>	13000	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 above ground weed coverage, where 0 = no reduction and 100 = no weeds present. Data pooled across years as treatment-by-year interaction was not significant.

^b Abbreviations: *P. macrostoma*, *Phoma macrostoma*; mu, macrocidin unit; WAT, weeks after treatment.

^c Combined broadleaf weeds is a visual evaluation of all broadleaf weeds in the treated plot, inclusive of those evaluated individually and any other dicot weeds present in the area.

^d Percent cover based on visual estimations of above ground turf coverage. Data pooled across years as treatment-by-year interaction was not significant.

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

Herbicide ^a	Rate	Unit	Yellow	Hairy	Ivyleaf
			woodsorrel	galinsoga	speedwell
			-----% Control ^b -----		
<i>P. macrostoma</i>	3250	mu m ⁻²	20	5	0
<i>P. macrostoma</i>	6500	mu m ⁻²	13	10	3
<i>P. macrostoma</i>	13000	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	412	g ai ha ⁻¹	95	87	79
LSD _{0.05}			9	8	7

^a *P. macrostoma*, *Phoma macrostoma*; mu, macrocidin unit

^b Percent control based on visual estimations of reductions in above ground plant biomass compared to 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 containing 0.026% dicamba, 0.605% 2,4-dichlorophenoxyacetic acid, 0.149% methylchlorophenoxypropionic acid

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

Herbicide	Rate ^a	Rate Unit	Henbit ^a		Marsh yellowcress		Flexuous bittercress		Dandelion		Common chickweed	
			2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
-----% Control ^b -----												
<i>P. macrostoma</i>	3250	mu m ⁻²	0	5	16	18	2	10	10	3	2	2
<i>P. macrostoma</i>	6500	mu m ⁻²	0	5	42	10	16	14	20	7	0	5
<i>P. macrostoma</i>	13000	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	412	g ai 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 *P. macrostoma*, *Phoma macrostoma*; mu, macrocidin unit.

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

^c Ready to use formulation containing 0.026% dicamba, 0.605% 2,4-dichlorophenoxyacetic acid, 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 days after application. Research conducted at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

Herbicide ^a	Rate	Unit	Common				Henbit ^b	Dandelion	Broadleaf weeds ^c
			chickweed		Sparrow vetch				
			2012	2013	2012	2013			
-----% Control ^d -----									
<i>P. macrostoma</i>	6500	mu m ⁻²	5	10	28	53	24	40	29
<i>P. macrostoma</i>	13000	mu m ⁻²	10	10	20	58	25	60	41
Thaxtomin A	190	g ai ha ⁻¹	78	13	20	23	50	34	25
Thaxtomin A	380	g ai ha ⁻¹	78	35	18	23	60	54	11
Weed B Gon ^e	412	g ai ha ⁻¹	85	93	90	80	32	50	90
LSD _{0.05}			9	14	28	13	14	21	21

^a Abbreviations: *P. macrostoma*, *Phoma macrostoma*; mu, macrocidin unit.

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

^c Broadleaf weeds is a visual evaluation of all broadleaf weeds in the treated plot, inclusive of those evaluated individually and any other dicot weeds present in the area.

^d Percent control based on visual estimations of reductions in above ground plant biomass compared to non-treated plots, where 0 = no reduction and 100 = complete plant mortality.

^e Ready to use formulation containing 0.026% dicamba, 0.605% 2,4-dichlorophenoxyacetic acid, 0.149% methylchlorophenoxypropionic acid

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 ^{ab}		
		4WAT ^c	10WAT	16WAT
		-----%-----		
Thaxtomin A	190	37	34	21
Thaxtomin A	380	60	66	46
Thaxtomin A	570	81	79	69
LSD _{0.05}		10	9	11

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

^b Data pooled across years as treatment-by-year effect was not significant.

^c Abbreviations: WAT, weeks after treatment.

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

Herbicide	Rate g ai ha ⁻¹	Henbit Control ^{ab}		
		4WAT ^c	4WAT2	16WAT2
		-----% ^c -----		
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 above ground weed coverage compared to nontreated plots, where 0 = no reduction and 100 = no weeds present.

^b Data pooled across years as treatment-by-year effect was not significant.

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

Part II

Efficacy of the Bioherbicide Thaxtomin A on Smooth Crabgrass and Annual Bluegrass and Safety in Cool-Season Turfgrasses

Abstract

Recent trends favoring organic and sustainable management practices in North America have led to an increasing demand for biological alternatives to traditional synthetic chemical pesticides. However, few bioherbicides are commercially available for selective weed control in turf. Thaxtomin A, a compound produced by the bacterium *Streptomyces scabies*, is a bioherbicide which has been reported to have PRE efficacy on broadleaf weeds, but efficacy of thaxtomin A on annual grassy weeds and safety to newly seeded cool-season turfgrasses have not been evaluated. Field experiments were conducted to evaluate PRE efficacy of thaxtomin A on smooth crabgrass and annual bluegrass. Thaxtomin A controlled smooth crabgrass through July but did not provide season-long control equivalent to the industry standard PRE herbicide. Initial applications of thaxtomin A at 380 g ai ha⁻¹ followed by two applications at 190 or 380 g ai ha⁻¹ at four week intervals provided season-long control of annual bluegrass similar to an industry standard PRE herbicide. Thaxtomin A reduced tall fescue and perennial ryegrass cover when applied 1 WBS, AS, and 1 WAS, but were safe at other application timings. Up to three applications of thaxtomin A at 380 g ai ha⁻¹ at four week intervals did not reduce perennial ryegrass cover. These results suggest that thaxtomin A can significantly suppress annual grassy weeds in turf, and that repeated applications of thaxtomin A can provide commercially acceptable control of annual bluegrass.

Nomenclature: thaxtomin A; annual bluegrass, *Poa annua* L., POAAN; smooth crabgrass, *Digitaria ischaemum* (Schreb.) Schreb. ex Muhl., DIGIS; tall fescue, *Lolium arundinaceum* (Schreb.) S.J. Darbyshire ‘Top Choice’, ‘Triple Threat’, FESAR; perennial ryegrass, *Lolium perenne* (L.) ‘Carly’, LOLPE.

Introduction

Weed control in turf is highly dependent upon the use of herbicides. Roughly 45 million kg of synthetic herbicides were applied to non-crop areas in the United States between 2000 and 2007 (Grube et al. 2011). As a consequence of this widespread use, pressure from environmental and human health advocacy groups has lead regulators to impose limitations on the use of synthetic chemical pesticides in many areas. Examples include Takoma Park, MD and Ogunquit, MN, which banned the use of all cosmetic pesticides in residential areas in 2013 and 2014, respectively (Feldman and Paul 2014; Pipkin 2013). A larger-scale ban was enacted in the state of New York in 2011, banning the use of all synthetic pesticides on school grounds (Grant 2011). These restrictions are even more widespread in Canada, where the provinces of Quebec and Ontario have banned the use of any synthetic pesticide for aesthetic purposes (Belair et al. 2010).

While governmental agencies and advocates for these bans have suggested integrated pest management (IPM) strategies and bioherbicides as alternatives to traditional chemical weed control strategies, there are few options available capable of providing commercially acceptable weed control (Chandler et al. 2011; Ghosheh 2005). In spite of these limitations, interest in organic and biologically-based pesticides has grown in recent years, with the number of US households utilizing only organic weed and insect control products increasing from 5 million in 2004 to 12 million in 2008 (Anonymous 2014). The global biopesticide market has seen similar growth, with projected sales exceeding \$1 billion in 2010 according to BCC Research, LLC's market research (Bailey et al. 2010).

Though interest in bioherbicides continues to grow, past attempts to commercialize bioherbicides have been met with limited success (Chandler et al. 2011; Pacanoski 2011). While many naturally occurring chemicals with herbicidal activity have been identified, it has been estimated that less than 10% of the bioherbicides developed for commercial use have been successfully registered and used with any regularity (Charudattan 2005). Obstacles for successful commercialization of bioherbicides typically include a narrow host range, which limits the products to a niche market, and fluctuations in efficacy based on environmental conditions at the time of application (Chandler et al. 2011; Johnson 1994). More recent attempts to develop commercially viable bioherbicides have focused on pathogens and biochemicals which affect a larger selection of weed species, or which provide control of specific, economically-important weeds with broad geographic distributions (Bourdôt et al. 2011; Boyetchko et al. 2009).

Annual bluegrass (*Poa annua*) and crabgrass (*Digitaria* spp.) are the most common and problematic winter annual and summer annual grassy weeds in turfgrass, respectively. Several species of crabgrass are common, with large crabgrass (*Digitaria sanguinalis*) and smooth crabgrass (*Digitaria ischaemum*) being the most prevalent (Kim et al. 2002). In the southern United States, annual bluegrass and crabgrass are ubiquitous weeds of turfgrass, with eight of nine states surveyed in 2012 reporting both among their most common turfgrass weeds (Webster 2012). Both annual bluegrass and crabgrass are well-adapted to regular

mowing, produce copious amounts of seeds, germinate rapidly when environmental conditions are conducive, and are considered highly undesirable by turf managers due to their light green colors and rough, uneven textures (Kim et al. 2002; Lush 1989). Presence of high populations of these weeds has a deleterious effect on both aesthetic and, in the case of sports turf, functional quality of the turf (Beard 1970; Kim et al. 2002; McCarty et al. 2011). Control of annual bluegrass and smooth crabgrass is typically accomplished through the use of both PRE and POST herbicides, but despite the availability of several effective herbicides crabgrass and annual bluegrass populations persist throughout much of the United States (Kim et al. 2002; McCarty et al. 2011). Management of annual bluegrass in newly-seeded or overseeded turfgrass systems has been highlighted as being particularly problematic, as many PRE herbicides which control annual bluegrass can also reduce turf quality or delay stand establishment when applied near the time of seeding (Cross et al. 2012; Lycan and Hart 2006; McCullough et al. 2011).

While several synthetic herbicides are labelled for control of annual bluegrass and crabgrass, few bioherbicides have been developed for this purpose. Corn gluten meal, a byproduct of the corn milling process, is an organic weed management option reported to selectively control annual grasses in turf, including annual bluegrass and crabgrasses (Bingaman and Christians 1995; Liu et al. 1994; McDade and Christians 2000). Recent studies suggest that corn gluten meal improved turf quality, but did not provide commercially acceptable weed control (Patton and Weisenberger 2011; Siva 2014; St. John and DeMuro 2013). The bacterium *Xanthomonas campestris* pv. *poannua*, which causes bacterial wilt of annual bluegrass, was also studied as a potential bioherbicide. In greenhouse and controlled environment tests annual bluegrass was well controlled, but in field experiments poor or variable control was observed (Johnson 1994; Zhou and Neal 1995). Chandramohan et al. (2002) reported that a mixture of fungi, including *Dreschlera gigantea*, *Exserohilum longirostratum* and *E. rostratum* effectively controlled several weedy grasses, including large crabgrass with little or no symptoms on turfgrasses. The research demonstrated that applications of *Dreschlera gigantea* mycelium in an oil emulsion resulted in 80% disease severity on large crabgrass 10 weeks after treatment, while the addition of ammonium sulfate was found to improve control to 100% (Shabana et al. 2010). Despite these results, development of this organism as a bioherbicide has not progressed. Bioherbicides capable of controlling annual grassy weeds are needed, as turf managers who wish to maintain their turf without traditional chemical herbicides have no effective commercially available alternatives.

Thaxtomin A is one of a class of compounds produced by the bacterium *Streptomyces scabies*, the causal organism of common scab disease in potatoes (King et al. 1992). Thaxtomin A has been shown to be phytotoxic to both broadleaf and grassy weeds, and possesses both PRE and POST activity (King et al. 2001). Fry and Loria (2002) observed seedling stunting, cellular hypertrophy, and cell wall lignification of onion seedlings treated with thaxtomin A. Multiple reports suggest that thaxtomin A is capable of inhibiting cellulose biosynthesis, though its exact molecular target has not yet been determined (Duke and Dayan 2011; Fry and Loria 2002). Synthetic herbicides which inhibit cellulose

biosynthesis, such as dichlobenil, isoxaben, and indaziflam, have been commercially available since the 1960s (Sabba and Vaughn 1999). Indaziflam, the most recently-introduced of the cellulose biosynthesis inhibiting herbicides, has become widely used in warm-season turfgrass and is capable of providing effective PRE control of annual grassy weeds like crabgrass and annual bluegrass (Brosnan et al. 2011; Brosnan et al. 2014; Henry et al. 2012). Additional data characterizing the herbicidal activity of thaxtomin A is scarce. Little is known about the ability of thaxtomin A to control most common weed species, including annual grasses, or its response to variable environmental conditions outside of laboratory and greenhouse experiments. In preliminary container experiments, PRE applications of thaxtomin A at 380 g ai ha⁻¹ caused a 23% reduction in large crabgrass and provided 100% control of annual bluegrass. These data suggest that thaxtomin A may have the potential to provide suppression of annual grassy weeds in turf.

In many circumstances traditional herbicides such as indaziflam cannot be utilized, such as in newly seeded cool-season turfgrasses or when warm-season turf is overseeded with a cool-season turfgrass. Thus, there exists a need for selective strategies, traditional or biologically, for annual bluegrass control in overseeded warm-season turfgrasses and seedling cool-season turf.

While thaxtomin A's reported herbicidal properties and mode of action make it a promising candidate for commercialization as a bioherbicide in turfgrass, further study into its activity on annual grasses, safety on turfgrass, and performance under field conditions is needed. The objectives of this study were to (1) evaluate the efficacy of PRE applications of thaxtomin A for control of smooth crabgrass and annual bluegrass, (2) determine appropriate seeding-to-treatment and treatment-to-seeding intervals for thaxtomin A safety on tall fescue and perennial ryegrass, and (3) evaluate the safety of thaxtomin A on creeping bentgrass (*Agrostis stolonifera*).

Materials and Methods

Field Sites and Experimental Design. Smooth crabgrass and annual bluegrass control experiments were conducted on low-maintenance common bermudagrass (*Cynodon dactylon*) fairways at Thorndale Country Club in Oxford, NC (36.31N, -78.59W) with a history of heavy smooth crabgrass and annual bluegrass infestations. The soil on this site was renovated during construction, with native soil being removed and backfilled with a fine sandy loam (Udorthents, loamy) with 0.71% humic matter and a pH of 4.7. Cool-season turf safety was evaluated in containers at the North Carolina State University Horticultural Field Lab in Raleigh, NC (35.79N, -78.7W). Tall fescue, perennial ryegrass, and creeping bentgrass safety experiments were conducted at the North Carolina State University Lake Wheeler Field Laboratory in Raleigh, NC (35.74N, -78.88W). The tall fescue and perennial ryegrass safety experiments were repeated at the North Carolina Department of Agriculture and Consumer Services Sandhills Research Station in Jackson Springs, NC (35.19N, -79.67W). The soil at the Lake Wheeler site was a Cecil sandy loam (fine, kaolinitic, thermic Typic Kanhapludults) with 1.19% humic matter and a pH of 5.8. The soil at the Sandhills site

was a Wakulla sand (siliceous, thermic Psammentic hapludults) with 0.41% humic matter and a pH of 6.2.

Treated plots were 1 by 2 m in size for efficacy studies at Thorndale Country Club and 1 m² in turfgrass safety tests at both the Lake Wheeler Field Laboratory and Sandhills Research Station. All treatments were arranged in randomized complete block designs with four replicates. Weed control was visually evaluated on a 0-10 scale where 0 = no control and 10 = 100% control. Control ratings were converted to percent control for presentation. Percent cover estimates were also recorded at each evaluation.

Smooth Crabgrass Control. Smooth crabgrass control experiments were initiated on March 28, 2013 and repeated on April 4, 2014. Single and multiple applications of thaxtomin A were compared. Treatments, doses, and application intervals are summarized in Table 1. In 2013, treatments included thaxtomin A at 190g ai ha⁻¹ applied one, two or three times at 4 week intervals; 380 g ai ha⁻¹ applied once or twice at 4 week intervals; 380 g ai ha⁻¹ followed by 190 g ai ha⁻¹ at four weeks; and 570 g ai ha⁻¹ applied once. These treatments were compared to an industry standard synthetic PRE herbicide, 1.68 kg ai ha⁻¹ pendimethalin (Pendulum®, BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709), and 96 kg ha⁻¹ corn gluten, each applied twice at eight week intervals. Additional treatments in 2014 included three applications of thaxtomin A at 380 g ai ha⁻¹ at four-week intervals, four applications of thaxtomin A at 190 and 380 g ha⁻¹ every four weeks, and an initial application of 380 g ha⁻¹ followed by two and three applications at 190 g ha⁻¹ at four week intervals. Experiments received no irrigation other than natural rainfall. In 2013, the experiment was mowed one to two times per week at a height of 1.9 cm, with clippings returned. In 2014, regular maintenance on the site ceased due to closure of the golf course and the experiment was mowed bi-weekly at a height of 1.9 cm, with clippings returned. The low maintenance conditions in 2014 resulted in poor bermudagrass health, reducing competition.

All treatments were applied with a CO₂ pressurized backpack sprayer equipped with two 8004 flat fan nozzles (TeeJet, Spraying Systems Co., North Ave. and Schmale Road, Wheaton, IL 60187) calibrated to deliver 280 L ha⁻¹. Crabgrass control and percent crabgrass cover was visually estimated at each evaluation. Evaluations were initiated in June when crabgrass had grown to sufficient size to be evaluated and were conducted monthly thereafter.

Annual Bluegrass Control. Annual bluegrass control experiments were conducted at Thorndale Country Club in Oxford, NC on a common bermudagrass fairway adjacent to the smooth crabgrass experiments. The experiment was initiated on September 18, 2013 and repeated on September 17, 2014. Treatments included thaxtomin A at 190 and 380 g ai ha⁻¹ applied once, twice, and three times at four-week intervals, a single application of thaxtomin A at 570 g ai ha⁻¹, and an initial application of thaxtomin A at 380 g ai ha⁻¹ followed by one and two applications at 190 g ai ha⁻¹. These treatments were compared to pendimethalin applied at the labeled rate of 1.68 kg ai ha⁻¹ and corn gluten at 96 kg ha⁻¹. The site received no irrigation other than natural rainfall. In 2013, the experiment was mowed one to two times

per week at a height of 1.9 cm, with clippings returned, until bermudagrass entered dormancy in November. In 2014, regular maintenance on the site ceased and the experiment was mowed bi-weekly at a height of 3.8 cm, with clippings returned, until bermudagrass entered dormancy in November.

Field plots in these experiments were 1 by 2 m in size in 2013 and 1 m² in size in 2014. All treatments were applied with a CO₂ pressurized backpack sprayer equipped with two 8004 flat fan nozzles calibrated to deliver 280 L ha⁻¹. Annual bluegrass control and percent annual bluegrass cover were visually estimated at each evaluation. Evaluations were initiated in November when annual bluegrass had emerged and grown to sufficient size to be evaluated and were conducted monthly thereafter.

Cool Season Turf Safety in Containers. A pilot study was conducted in containers to evaluate the safety of thaxtomin A applied to tall fescue and perennial ryegrass at seeding and after seeding. Seeds were sown into 10 cm square pots (0.95 L volume) containing a peat-based greenhouse potting mix (Fafard® 4P Potting Soil Mix, Sun Gro Horticulture, 770 Silver Street, Agawam MA 01001). Pots were seeded weekly with ‘Carly’ perennial ryegrass between March 14, 2014 and April 29, 2014. Separate pots were seeded weekly with ‘Triple Threat’ tall fescue between April 11, 2014 and May 29, 2014, with the last seeding made on the day of thaxtomin application. Seeding was done by hand at a rate of approximately 100 seeds per pot. After seeding, pots were maintained in a covered shade house, watered as needed and clipped weekly at a 5 cm height. Treatments included thaxtomin A at 190, 380 and 570 g ai ha⁻¹ applied to pots at seeding (AS) and one, two, four, and six weeks after seeding (WAS). Applications were made on perennial ryegrass on April 29, 2014, and applications were made on tall fescue on May 29, 2014.

Evaluations were conducted weekly and included visual estimates of turf injury based on a 0-10 scale with 0 = no injury and 10 = complete turf mortality (data were converted to percent injury for presentation). At the final evaluation four weeks after applications, above ground fresh weights were collected. Results from these tests were used to select appropriate application intervals in subsequent field experiments.

Tall Fescue Safety. The safety of thaxtomin A applied before seeding, at seeding and after seeding tall fescue was evaluated at both field sites. Areas used in these experiments received a broadcast application of glyphosate at 1 kg ai ha⁻¹ two weeks prior to initial thaxtomin A applications to remove existing vegetation. Treatments included thaxtomin A at 380 and 570 g ai ha⁻¹ applied four, two, and one week before seeding (WBS), at seeding (AS), and one, two, four and six weeks after seeding (WAS). PRE treatment timings were chosen based on prior PRE experiments utilizing thaxtomin A, including the smooth crabgrass and annual bluegrass experiments described above. POST treatment timings were chosen based on the experiments evaluating the safety of POST applications of thaxtomin A to cool season turfgrasses in containers, also described above. Treatments were applied using a CO₂ pressurized backpack sprayer equipped with 8006 flat fan nozzles calibrated to deliver 467 L ha⁻¹.

Tall fescue was seeded at both locations on September 19, 2014. ‘Triple threat’ blend turf-type tall fescue (Southern Seeds Inc., 10680 E Finch Ave, Middlesex, NC 27557) was slit- and broadcast seeded at 391 kg ha⁻¹. A slit-seeder (Dethatcher/seedler on 5 cm centers, 55.9 cm wide, BlueBird S22 Seeder, 9335 Harris Corners Parkway, Charlotte, NC 28269) was used to slit-seed in at least four directions; a broadcast spreader was then used to spread remaining seed uniformly. Seeded areas were amended with an 18-24-12 (N-P₂O₅-K₂O) slow release granular fertilizer (Lesco ® Professional Starter Fertilizer, Turf Care Supply Co., 50 Pearl Road, Brunswick, OH 44224) at a rate of 48.8 kg N ha⁻¹. Experiments were fertilized a second time on November 14, 2014 using a 16-4-8 (N-P₂O₅-K₂O) granular fertilizer (Green Eagle Professional Turf Fertilizer, Camp Chemical Corporation, 200 Hester Street, Roxboro, NC 27573) at a rate of 48.8 kg N ha⁻¹. After seeding both locations received 0.25 cm of overhead irrigation two times daily until germination had occurred, after which they received irrigation as-needed to maintain turfgrass growth. Following establishment, tall fescue was mowed at 5 cm one to two times per week using a rotary mower, with clippings removed from the site after mowing.

Beginning four weeks after seeding, evaluations were conducted bi-weekly and included visual estimates of percent turf cover on a 0 (no turf present) to 100% (complete turf cover) scale, and percent injury on a 0-10 scale where 0 = no injury and 10 = complete turf mortality (data were converted to percentage for presentation). At the final evaluation 10 weeks after seeding, four weeks after final applications, visual estimates of percent turf cover and turf quality on a 1-9 scale were collected, along with estimations of turf establishment using line-intersect analysis as described by Hoyle et al. (2013) using a 1m by 1m frame at 10 cm spacing. The frame was placed in the center of the treated plots and presence or absence of turf at each line intersect was recorded.

Perennial Ryegrass Safety. The safety of thaxtomin A applied before seeding, at seeding and after seeding perennial ryegrass in dormant bermudagrass turf was evaluated at both field sites. Treatments in this experiment were identical to those described above in the tall fescue safety experiment, with two additional treatments added: two applications of thaxtomin A at 380 g ai ha⁻¹ applied two weeks before seeding and two weeks after seeding, and three applications of thaxtomin A at 380 g ai ha⁻¹ applied two weeks before seeding, two weeks after seeding, and six weeks after seeding.

‘Carly’ perennial ryegrass (Southern Seeds Inc., 10680 E Finch Ave, Middlesex, NC 27557) was surface seeded at a rate of 391 kg ha⁻¹ onto stands of established ‘TifSport’ bermudagrass on October 3, 2014. Immediately afterwards, seeded areas were amended with an 18-24-12 (N-P₂O₅-K₂O) slow release granular fertilizer at a rate of 48.8 kg N ha⁻¹. Experiments were fertilized a second time on November 14, 2014 using a 16-4-8 (N-P₂O₅-K₂O) granular fertilizer at a rate of 48.8 kg N ha⁻¹. After seeding both locations received 0.25 cm of overhead irrigation two times daily until germination had occurred, after which they received irrigation as-needed to maintain sufficient soil moisture. Following establishment, perennial ryegrass was mowed at 5 cm 1-2 times per week using a reel mower, with clippings removed

from the site after mowing. Application and evaluation methods were identical to those used in the tall fescue safety experiment, described above.

Creeping Bentgrass Safety. An experiment was conducted at the Lake Wheeler site in 2013 to evaluate the safety of thaxtomin A when applied to creeping bentgrass (50/50 blend of 'A1' and 'A4') on a USGA-spec sand putting green. Treatments included thaxtomin A at 190, 380, and 570 g ai ha⁻¹, and sequential applications of 190 and 380 g ai ha⁻¹ each applied twice at a four week interval. Initial treatments were on October 18, 2013; sequential treatments were applied on November 15, 2013. Creeping bentgrass was maintained at a 0.32 cm mowing height using a reel mower, with clippings removed. The green was vertically mowed at a depth of 0.6 cm and then topdressed with sand on November 8, one week prior to the second application. Evaluations were conducted beginning one week after the initial application, and weekly thereafter for eight weeks. Afterward, evaluations were made monthly. Injury was evaluated visually on a 0-10 scale, with 0 = no injury and 10 = 100% necrosis. The experiment was not repeated due to the significant turfgrass injury observed.

Statistical Analysis. Data collected from all experiments were subjected to analysis of variance using Proc GLM in SAS (SAS 9.4, SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC 27513) to test for both main treatment effects and interactions. Where treatment effects were determined to be significant, treatment means were separated using Fisher's protected least significant difference method at a significance level of 0.05. When the interaction between year or location and treatment effect was not significant, data were pooled across years or locations. When there was a significant interaction between years, data are presented by year or by location.

Results and Discussion

Smooth Crabgrass Control. Treatment-by-year interactions were significant for all rating dates, thus data are presented by year. All treatments, including pendimethalin, provided better control in 2013 compared to 2014 at all rating dates. This may be explained by the lack of regular maintenance on the site in 2014. Healthy, dense turf receiving regular maintenance and fertility inputs is generally associated with lower weed populations (DeBels 2012; Elfort et al. 2008). In 2014, the site was mowed less frequently and received no inputs, resulting in poor bermudagrass cover and higher weed competition. Data for percent control and percent cover evaluations were negatively correlated ($R = -0.966$ and -0.935 in 2013 and 2014, respectively). Percent control data exhibited a lower coefficient of variability than percent cover data. Thus, for brevity, only percent control data are presented herein.

Smooth crabgrass control improved with increased dose of thaxtomin A from 190 to 570 g ai/ha, and longevity of weed control was improved by multiple applications. In 2013, thaxtomin A at 380 g ai ha⁻¹ applied twice provided 85 and 80% control of crabgrass in June and July, respectively, equivalent to pendimethalin (Table 1). Initial applications of thaxtomin A at 380 g ai ha⁻¹ followed by one and two applications of thaxtomin A at 190 g ai ha⁻¹ also provided 63-70% control of crabgrass through July, but were not equal to

pendimethalin (Table 1). In August, no thaxtomin A treatment was equal to pendimethalin, and the only treatments which provided greater than 50% crabgrass control were pendimethalin (92%) and two applications of thaxtomin A at 380 g ai ha⁻¹ (63%) (Table 1).

In 2014, thaxtomin A at 380 g ai ha⁻¹ applied three and four times provided 74% and 81% control of crabgrass in June, which was equivalent to the pendimethalin (Table 1). Unlike in 2013, two applications of thaxtomin A at 380 g ai ha⁻¹ did not provide control equal to the pendimethalin. In July, the only treatment which provided control equal to pendimethalin was four applications of thaxtomin A at 380 g ai ha⁻¹, which controlled crabgrass 68%. Control continued to decline through August. In August, four applications of thaxtomin A provided only 47% crabgrass control, but this was still statistically equivalent to pendimethalin which provided 68% control at this rating date. At the final evaluation in September, four applications of thaxtomin A at 380 g ai ha⁻¹ provided only 37% control of crabgrass and was no longer equal to pendimethalin (Table 1).

These data suggest that while thaxtomin A controls smooth crabgrass and is capable of providing smooth crabgrass suppression through much of the summer, four applications of thaxtomin A at 380 g ai ha⁻¹ were not sufficient for season-long control in these tests. Additional applications of thaxtomin A may improve control. Differences in the results observed in 2013 and 2014 also suggest that control of smooth crabgrass with thaxtomin A may be greater in healthier turfgrass under regular maintenance.

Annual Bluegrass Control. Treatment-by-year interactions were significant for the evaluations conducted in November and January, and so data are presented by year. Treatment-by-year interactions were not significant for March and April evaluations, thus these data are pooled across years for analysis and presentation. In November and January, control of annual bluegrass was generally similar or better in 2013 than in 2014. Regular maintenance on the trial site ceased prior to the final evaluations being made in 2013, and continued through the 2014 experiment. Thus, it is likely that the results observed in March and April were similar in 2013 and 2014 due to the similarity in conditions between the two years. Data for percent control and percent cover were negatively correlated ($R = -0.985$ and -0.986 in 2013 and 2014, respectively), thus only percent control data are presented.

In November 2013, thaxtomin A applied once, twice, and three times at 380 g ai ha⁻¹ controlled annual bluegrass 75-80%, equal to control with pendimethalin (Table 2). An initial application of thaxtomin A at 380 g ai ha⁻¹ followed by two applications at 190 g ai ha⁻¹ also provided 77% control, also equivalent to pendimethalin. Two applications of thaxtomin A at 190 g ai ha⁻¹ also provided annual bluegrass control equal to pendimethalin (62%) (Table 2). When evaluated the following January, only treatments including an initial application of thaxtomin A at 380 g ai ha⁻¹ provided control equal to pendimethalin. Thaxtomin A at 380 g ai ha⁻¹ followed by two applications at 190 or 380 g ai ha⁻¹ provided 77-90% control of annual bluegrass, equal to pendimethalin (Table 2).

In November 2014, two and three applications of thaxtomin A at 380 g ai ha⁻¹ provided 83% and 87% control of annual bluegrass, equal to pendimethalin. Unlike in 2013, a single application of thaxtomin A at 380 g ai ha⁻¹ did not provide similar control into November, likely because annual bluegrass emergence occurred earlier in 2014 (Table 2). By January, all treatment regimens starting with 190 g ai ha⁻¹ thaxtomin A were less effective compared to pendimethalin. Treatment regimens with an initial thaxtomin A dose of 380 followed by one or two applications at 190 or 380 g ai ha⁻¹ controlled annual bluegrass equivalent to pendimethalin (Table 2).

In March and April of both years, the only treatments which provided annual bluegrass control equal to pendimethalin were thaxtomin A applied three times at 380 g ai ha⁻¹, and thaxtomin A applied once at 380 g ai ha⁻¹ followed by two applications at 190 g ai ha⁻¹ (69% control in March, 67-71% control in April). A decline in annual bluegrass control is often observed in March following the application of PRE herbicides like pendimethalin in the fall, as annual bluegrass growth and germination resume in conjunction with increased herbicide dissipation after temperatures rise in the spring (Park et al. 2002).

These data suggest that thaxtomin A can provide season-long annual bluegrass control similar to that of an industry standard synthetic PRE herbicide. Multiple applications at regular intervals will be necessary to maintain control. In these experiments, an initial PRE application of 380 g ai ha⁻¹ thaxtomin A followed by two applications at 190 to 380 g ai ha⁻¹ was required to maintain control into the spring. Further research is required to determine whether these application regimes will provide sufficient control in other turfgrass systems and under different environmental conditions.

Cool Season Turf Safety in Containers. Visual ratings of percent injury were negatively correlated with above-ground fresh weights ($R = -0.99$ and -0.82 for fescue and perennial ryegrass, respectively). Treatment timing, rate, and timing by rate interaction were significant for both perennial ryegrass and tall fescue.

Thaxtomin A caused greater injury to tall fescue than perennial ryegrass (Table 3). Injury to both species increased as dose increased and decreased as the time between seeding and application increased. When applied AS, tall fescue was injured 40% by thaxtomin A at 190 g ai ha⁻¹. When applied 1 and 2 WAS this rate caused only 10% injury, and applications made 4 and 6 WAS caused no injury (Table 3). At 380 g ai ha⁻¹, thaxtomin A caused 60% injury to tall fescue when applied AS. 53% 1 WAS, and 35% 2 WAS. When applied 4 WAS, 380 g ai ha⁻¹ thaxtomin A caused only 8% injury to tall fescue, and did not cause injury when applied 6 WAS (Table 3). When applied at 570 g ai ha⁻¹, thaxtomin A caused 89% injury to tall fescue AS. Injury decreased to 55% and 43% at 1 WAS and 2 WAS, respectively. When applied 4 WAS, 570 g ai ha⁻¹ thaxtomin A caused 13% injury, and when applied at 6 WAS this rate caused only 2% injury (Table 3).

When applied at 190 g ai ha⁻¹, thaxtomin A caused $\leq 1\%$ injury to perennial ryegrass regardless of timing (Table 3). At 380 g ai ha⁻¹, thaxtomin A caused 5% injury to perennial

ryegrass when applied AS, which decreased to 3% at 1 WAS and 1% at 2 WAS. When applied 4 and 6 WAS, thaxtomin A did not injure perennial ryegrass at 380 g ai ha⁻¹ (Table 3). An application of thaxtomin A at 570 g ai ha⁻¹ AS caused 15% injury to perennial ryegrass, which decreased to 7% when applied 1 WAS and 5% when applied 2 WAS. When applied 4 WAS and 6 WAS, thaxtomin A at 570 g ai ha⁻¹ caused 1% injury to perennial ryegrass (Table 3).

Tall Fescue Safety. Treatment-by-location interactions were non-significant at all evaluation dates, thus data were pooled across locations for presentation. Treatment timing and application rate were significant at all rating dates, but no interaction between timing and rate was detected.

When applied four WBS, neither rate of thaxtomin A reduced tall fescue cover compared to non-treated turf. When applied two WBS, thaxtomin A at 570 g ai ha⁻¹ significantly reduced tall fescue cover at all rating dates, resulting in a 14% reduction at the end of the experiment (Table 4). Thaxtomin A at 380 g ai ha⁻¹ did not reduce tall fescue cover when applied two WBS. Applied one WBS, AS and one WAS both 380 and 570 g ai ha⁻¹ thaxtomin A reduced tall fescue cover by 28- 42%, 60-72%, and 22-40%, respectively (Table 4). When applied two WAS, thaxtomin A at 570 g ai ha⁻¹ did not immediately reduce tall fescue cover, but within four weeks of the application turf injury was observed. By the end of the experiment, tall fescue cover was reduced 13% by this treatment (Table 4). Thaxtomin A at 380 g ai ha⁻¹ applied two WAS did not cause injury. When applied four and six WAS, neither rate of thaxtomin A significantly reduced tall fescue cover (Table 4). Turfgrass injury observed in these experiments manifested as stunting and eventual death, similar to the typical symptoms of cellulose biosynthesis inhibition observed by Fry and Loria (2002).

These data suggest that thaxtomin A at 380 g ai ha⁻¹ can be safely applied to tall fescue two WBS, and that applications made two WAS and beyond should not significantly reduce tall fescue cover. At 570 g ai ha⁻¹, thaxtomin A can be safely applied to tall fescue four WBS and four WAS.

Perennial Ryegrass Safety. Treatment-by-location interactions were significant at all evaluation dates, thus data are presented by location. Treatment timing and application rate was also significant at all evaluation dates. General trends in reductions in perennial ryegrass cover were similar between the two sites, but perennial ryegrass cover was higher on average at the Sandhills site.

When evaluated four WAS, neither dose of thaxtomin A applied four WBS reduced perennial ryegrass cover. When applied two WBS, thaxtomin A at 570 g ai ha⁻¹ did not reduce perennial ryegrass cover at the Lake Wheeler site but at the Sandhills site percent cover was 17% less than in non-treated plots. Applications of thaxtomin A one WBS reduced perennial ryegrass cover 30-36% at the Lake Wheeler site and 22-43% at the Sandhills site. When applied AS, thaxtomin A resulted in 60-80% less perennial ryegrass cover at the Lake Wheeler site and 34-67% less cover at the Sandhills site (Table 5). Applications of thaxtomin A made one WAS also reduced perennial ryegrass cover at both locations, 49-60% at the

Lake Wheeler site and 39-52% at the Sandhills site. When applied two WAS, thaxtomin A at 570 g ai ha⁻¹ perennial ryegrass cover was 14% less than the nontreated at the Sandhills site, but did not reduce perennial ryegrass cover at the Lake Wheeler site (Table 5).

At the final evaluation ten WAS, the only treatments which reduced perennial ryegrass cover at either location were both rates of thaxtomin A applied one WBS, AS, and one WAS. Perennial ryegrass cover was reduced by thaxtomin A at 380 g ai ha⁻¹ applied one WBS at the Lake Wheeler site, but was not at the Sandhills site, while thaxtomin A at 380 g ai ha⁻¹ applied AS and one WAS reduced perennial ryegrass cover at both sites. At 570 g ai ha⁻¹, thaxtomin A reduced perennial ryegrass when applied one WBS, AS, and one WAS. Perennial ryegrass cover was unaffected by the sequential applications of 380 g ai ha⁻¹ thaxtomin A made two WBS and two WAS, or by three applications made two WBS, two WAS, and six WAS (Table 4).

Creeping Bentgrass Safety. All thaxtomin A treatments caused significant injury to creeping bentgrass after a single application, and injury increased as thaxtomin A dose increased (Table 6). Thaxtomin A at 190 g ai ha⁻¹ caused 20% injury to creeping bentgrass when evaluated four WAT, though the severity of injury had decreased to 13% by 16 WAT (Table 6). When applied at 380 g ai ha⁻¹, thaxtomin A injured creeping bentgrass 38% four WAT. Creeping bentgrass recovery was less noticeable at this rate. When evaluated 16 WAT, creeping bentgrass treated with thaxtomin A at 380 g ai ha⁻¹ still exhibited 33% injury (Table 6). Creeping bentgrass treated with thaxtomin A at 570 g ai ha⁻¹ was injured 53-63% and did not show visible signs of recovery by 16 WAT (Table 6). Sequential applications of thaxtomin A at both 190 and 380 g ai ha⁻¹ increased creeping bentgrass injury. Following a second application of thaxtomin A at 190 g ai ha⁻¹ creeping bentgrass injury increased from 20% to 40%. At 380 g ai ha⁻¹, a second application of thaxtomin A increased creeping bentgrass injury from 43% to 68% (Table 6). By 16 WAT, creeping bentgrass had not recovered from two applications of thaxtomin A at 190 or 380 g ai ha⁻¹, with injury still 38% and 65%, respectively (Table 6).

These data suggest that cool-season turfgrass species vary in their responses to thaxtomin A applications. Thaxtomin A will not reduce perennial ryegrass cover when applied 2 WBS or 2WAS, and that thaxtomin A is generally less injurious to perennial ryegrass than tall fescue (Figure 1). Creeping bentgrass mowed at 0.32 cm did not tolerate applications of thaxtomin A at any dose tested. Additionally, these data suggest that perennial ryegrass is tolerant of repeated applications of thaxtomin A when made 2 WBS, 2 WAS, and 6 WAS at 380 g ai ha⁻¹. These findings, taken in conjunction with the annual bluegrass control following three applications of thaxtomin A at this rate and interval, suggest that thaxtomin A could be used to provide PRE control of annual bluegrass while also allowing the safe establishment of over-seeded perennial ryegrass. While thaxtomin A may not provide commercially acceptable control of smooth crabgrass in a low-maintenance turf system, these results demonstrate significant reductions in smooth crabgrass cover. Further research is warranted

to evaluate strategies for improved longevity of control or turfgrass management strategies that might enhance thaxtomin A efficacy on summer annual grass weed control.

Literature Cited

- Anonymous (2014) Lawn and Garden Services Industry Report.
<http://business.highbeam.com/industry-reports/agriculture/lawn-garden-services>
Accessed May 28, 2015
- Bailey KL, Boyetchko SM, Längle T (2010) Social and economic drivers shaping the future of biological control: A Canadian perspective on the factors affecting the development and use of microbial biopesticides. *Biol Control* 52(3):221-9
- Beard JB (1970). An ecological study of annual bluegrass. *USGA Green Sect Rec* 8(2):13–18
- Bingaman BR, Christians NE (1995) Greenhouse screening of corn gluten meal as a natural product for broadleaf and grass weeds. *HortScience* 30(6):1256-1259
- Bourdôt GW, Saville DJ, Jong MD (2011) Evaluating the environmental safety of broad-host-range bioherbicides. *Pest Technol* 5:34-40
- Boyetchko SM, Bailey KL, De Clerck-Floate RA (2009) Current biological weed control agents - their adoption and future prospects. *Prairie Soils and Crops* 2:6.
- Brosnan JT, McCullough PE, Breeden GK (2011) Smooth crabgrass control with indaziflam at various spring timings. *Weed Technol* 25:363-366
- Brosnan JT, Reasor EH, Vargas JJ, Breeden GK, Kopsell DA, Cutule MA, Mueller TC (2014) A putative prodiamine-resistant annual bluegrass (*Poa annua*) population is controlled by indaziflam. *Weed Sci* 62:138-144.
- Chandler D, Bailey AS, Tatchell GM, Davidson G, Greaves J, Grant WP (2011) The development, regulation and use of biopesticides for integrated pest management. *Philos Trans R. Soc, B* 366(1573):1987-98
- Chandramohan S, Charudattan R, Sonoda RM, Singh M (2002) Field evaluation of a fungal pathogen mixture for the control of seven weedy grasses. *Weed Sci* 50(2):204-213
- Charudattan R (2005) Ecological, practical, and political inputs into selection of weed targets: What makes a good biological control target? *Biol Control* 35(3): 183-196
- Cross RB, McCarty LB, Estes AG, Sharp JL, Toler JE (2012) Annual bluegrass control in overseeded golf course fairways when mitosis-inhibiting herbicides are not effective. *Appl Turfgrass Sci* DOI:10.1094/ATS-2012-0523-01-RS
- DeBels BT, Griffith SE, Kreuser WC, Melby ES, Soldat DJ (2012) Evaluation of mowing height and fertilizer application rate on quality and weed abundance of five home lawn grasses. *Weed Technol* 26:828-831.

- Duke SO, Dayan FE (2011) Modes of action of microbially-produced phytotoxins. *Toxins* 3(8):1038-1064
- Elfort EMA, Tardif FJ, Robinson DE, Lyons EM (2008) Effect of perennial ryegrass overseeding on weed suppression and sward composition. *Weed Technol* 22:231-239
- Feldman J, Paul K (2014) Ogunquit leads the way with ordinance banning pesticides. <http://bangordailynews.com/2014/11/16/opinion/contributors/ogunquit-leads-the-way-with-ordinance-banning-pesticides> Accessed May 28, 2015
- Fry BA, Loria R (2002) Thaxtomin A: evidence for a plant cell wall target. *Physiol Mol Plant Pathol* 60(1): 1-8
- Ghosheh HZ (2005) Constraints in implementing biological weed control: a review. *Weed Biol Manage* 5:83-92
- Grant J (2011). The child safe playing fields act: NY's ban on pesticide use on school and day care center grounds. http://www.hort.cornell.edu/turf/pdfs/school_ban_CUTT_2011.pdf. Accessed May 28, 2015
- Henry GM, Brosnan JT, Breeden GK, Cooper T, Beck LL, Straw CM (2012) Indaziflam programs for weed control in overseeded bermudagrass turf. *HortTechnology* 22(6):774-777
- Hoyle JA, Yelverton FH, Gannon TW (2013) Evaluation multiple rating methods utilized in turfgrass weed science. *Weed Technol* 27:362-368
- Johnson BJ (1994) Biological control of annual bluegrass with *Xanthomonas campestris* pv. *poannua* in bermudagrass. *Hort Sci* 29:659-662
- Kim T, Neal JC, Ditomaso JM, Rossi FS (2002) A survey of weed scientists' perception on the significance of crabgrasses (*Digitaria spp.*) in the United States. *Weed Technol* 16:239-242
- King RR, Lawrence CH, Calhoun LA (1992) Chemistry of phytotoxins associated with *Streptomyces scabies*, the causal organism of potato common scab. *J Agric Food Chem* 40(5):834-837
- King RR, Lawrence CH, Gray JA (2001) Herbicidal properties of the thaxtomin group of phytotoxins. *J Agric Food Chem* 49(5):2298-2301
- Lush WM (1989) Adaptation and differentiation of golf course populations of annual bluegrass. *Weed Sci* 37:54-59
- Lycan DW, Hart SE (2006) Cool-season turfgrass reseeding intervals for bispyribac-sodium. *Weed Technol* 20:526-629
- McCarty LB, Murphy TR, Boyd J, Yelverton F (2011) Turfgrass weeds. Pages 663-704 in LB McCarty ed. *Best Golf Course Management Practices*. 3rd ed. Upper Saddle River NJ: Pearson Education

- McCullough PE, Brosnan JT, Breeden GK (2011) Tall fescue and perennial ryegrass reseeding intervals for amicarbazone. *HortScience* 46(4):648-650
- Pacanoski Z (2011) Bioherbicides - a real or virtual measure for efficient weed control? *Herbologia* 12(3): 103-114
- Park N, Suto Y, Miura Y, Nakatani N, Iori S, Ogasawara M (2002) Annual bluegrass (*Poa annua* L.) control in bentgrass (*Agrostis palustris* Huds.) green with sequential applications of bispyribac-sodium combined with dinitroanilines. *Weed Biol Manag* 2:159-162
- Pipkin W (2013) Takoma Park bans 'cosmetic' lawn pesticides.
http://www.bayjournal.com/article/takoma_park_bans_cosmetic_lawn_pesticides.
Accessed May 28, 2015
- Sabba RP, Vaughn KC (1999) Herbicides that inhibit cellulose biosynthesis. *Weed Sci* 47:757-763
- Shabana YM, Stiles CM, Charudattan R, Tabl AH (2010) Evaluation of bioherbicidal control of tropical signalgrass, crabgrass, smutgrass, and torpedograss. *Weed Technol* 24(2):165-172.
- Zhou T, Neal JC (1995) Annual bluegrass (*Poa annua*) control with *Xanthomonas campestris* pv. *poannua* in New York State. *Weed Technol* 9(1):173-177

Table 1. PRE control of smooth crabgrass with thaxtomin A and pendimethalin. Research conducted at Thorndale Country Club (Oxford, NC).

Herbicide	Rate at each application ^a				Percent control of smooth crabgrass							
					June		July		August		September	
	1	2	3	4	2013	2014	2013	2014	2013	2014	2013	2014
	----- g ai ha ⁻¹ -----				-----%-----							
Thaxtomin A	190	0	0	0	0	12	0	17	0	10	-----	5
Thaxtomin A	380	0	0	0	35	15	35	7	30	10	-----	5
Thaxtomin A	570	0	0	0	63	10	48	17	33	10	-----	0
Thaxtomin A	190	190	0	0	45	33	40	27	30	10	-----	10
Thaxtomin A	380	190	0	0	70	45	63	20	43	15	-----	10
Thaxtomin A	380	380	0	0	85	50	80	35	63	28	-----	13
Thaxtomin A	190	190	190	0	70	27	70	27	50	0	-----	0
Thaxtomin A	380	190	190	0	-----	40	-----	20	-----	0	-----	5
Thaxtomin A	380	380	380	0	-----	74	-----	47	-----	25	-----	15
Thaxtomin A	190	190	190	190	-----	37	-----	40	-----	20	-----	5
Thaxtomin A	380	190	190	190	-----	53	-----	30	-----	13	-----	10
Thaxtomin A	380	380	380	380	-----	81	-----	68	-----	47	-----	37
Pendimethalin	1680	0	1680	0	97	84	97	76	92	68	-----	60
LSD _{0.05}					17	17	22	28	20	23	-----	14

^a Sequential treatments were applied at 4-week intervals. Additional sequential treatments were added to the experiment in 2014.

Table 2. PRE control of annual bluegrass with thaxtomin A and pendimethalin. Research conducted at Thorndale Country Club (Oxford, NC).

Herbicide	Rate at each application ^a			Percent control of annual bluegrass ^b					
	1	2	3	November		January		March	April
				2013	2014	2013	2014		
----- g ai ha ⁻¹ -----			----- % -----						
Thaxtomin A	190	0	0	40	15	37	8	10	12
Thaxtomin A	380	0	0	75	23	77	5	25	29
Thaxtomin A	190	190	0	62	67	32	40	19	21
Thaxtomin A	380	190	0	55	70	67	67	40	40
Thaxtomin A	380	380	0	77	83	82	55	48	37
Thaxtomin A	190	190	190	42	30	50	13	22	16
Thaxtomin A	380	190	190	77	80	90	67	69	67
Thaxtomin A	380	380	380	80	87	82	67	69	71
Pendimethalin	1680	0	1680	86	77	92	63	69	73
LSD _{0.05}				26	21	16	23	17	17

^a Sequential treatments were applied at 4-week intervals. Additional sequential treatments were added to the experiment in 2014.

^b Treatment by year interactions were significant for November and January but not for March and April evaluations; therefore, data for March and April evaluations were merged for analysis and presentation.

Table 3. Effects of post-seeding applications of thaxtomin A on tall fescue and perennial ryegrass establishment in containers four weeks after application. Research conducted at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

Timing	Rate g ai ha ⁻¹	Injury	
		FESAR -----%	LOLPE -----
AS	190	40	0
AS	380	60	5
AS	570	89	15
1 WAS	190	10	0
1 WAS	380	53	3
1 WAS	570	55	7
2 WAS	190	10	0
2 WAS	380	35	1
2 WAS	570	43	5
4 WAS	190	0	1
4 WAS	380	8	0
4 WAS	570	13	1
6 WAS	190	0	0
6 WAS	380	0	0
6 WAS	570	2	1
LSD _{0.05}		10	4
ANOVA P values			
Timing		<0.0001	<0.0001
Rate		<0.0001	<0.0001
Timing X Rate		<0.0001	0.0001

^a Abbreviations: FESAR, tall fescue; LOLPE, perennial ryegrass; AS, at seeding; WAS, weeks after seeding.

Table 4. Effects of pre- and post-seeding applications of thaxtomin A on tall fescue establishment. Research conducted at the North Carolina State University Lake Wheeler Field Laboratory (Raleigh, NC) and the North Carolina Department of Agriculture and Consumer Services Sandhills Research Station (Jackson Springs, NC).

Timing	Rate	Tall fescue cover ^{ab}				
		2WAS	4WAS	6WAS	8WAS	10WAS
		-----%-----				
g ai ha ⁻¹						
Non-treated	N/A	48	76	79	78	83
4WBS	380	54	77	76	81	85
4WBS	570	44	79	79	81	86
2WBS	380	42	71	70	70	79
2WBS	570	30	57	59	59	69
1WBS	380	10	27	37	40	55
1WBS	570	8	19	25	26	41
AS	380	5	9	12	13	23
AS	570	2	2	5	6	11
1WAS	380	25	37	45	46	61
1WAS	570	21	30	33	30	43
2WAS	380	-----	73	72	79	84
2WAS	570	-----	82	55	61	70
4WAS	380	-----	-----	77	81	87
4WAS	570	-----	-----	78	80	76
6WAS	380	-----	-----	-----	81	86
6WAS	570	-----	-----	-----	69	74
LSD _{0.05}		8	12	10	10	11
ANOVA P values						
Timing		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Rate		0.0042	0.0112	0.0022	<0.0001	<0.0001
Timing X Rate		---NS---	---NS---	---NS---	---NS---	---NS---

^a Abbreviations: AS, at seeding; WAS, weeks after seeding; WBS, weeks before seeding; NS, not significant.

^b Data were pooled across locations as treatment-by-location interaction was not significant.

Table 5. Effects of pre- and post-seeding applications of thaxtomin A on perennial ryegrass establishment in over-seeded bermudagrass turf. Research conducted at the North Carolina State University Lake Wheeler Field Laboratory (Raleigh, NC) and the North Carolina Department of Agriculture and Consumer Services Sandhills Research Station (Jackson Springs, NC).

Timing	Rate g ai ha ⁻¹	Percent cover of perennial ryegrass ^a			
		4WAS		10WAS	
		LW	SH	LW	SH
		-----%			
Non-treated	N/A	86	84	91	95
4WBS	380	84	80	89	97
4WBS	570	87	80	90	97
2WBS	380	79	72	91	94
2WBS	570	79	67	86	87
1WBS	380	56	62	75	94
1WBS	570	50	41	57	77
AS	380	26	50	47	82
AS	570	6	17	25	65
1WAS	380	37	45	46	80
1WAS	570	26	32	42	66
2WAS	380	82	76	95	96
2WAS	570	81	70	92	92
4WAS	380	-----	-----	92	93
4WAS	570	-----	-----	89	91
6WAS	380	-----	-----	86	96
6WAS	570	-----	-----	95	92
2WBS fb 2WAS	380	71	70	84	91
2 WBS fb 2 WAS fb 6 WAS	380	-----	-----	87	93
LSD _{0.05}		13	13	14	10
ANOVA P values					
Timing		<0.0001	<0.0001	<0.0001	<0.0001
Rate		0.0374	<0.0001	0.0225	0.0004
Timing X Rate		-NS-	0.0112	---NS---	---NS---

^a Abbreviations: AS, at seeding; WAS, weeks after seeding; WBS, weeks before seeding; LW, Lake Wheeler field laboratory; SH, Sandhills research station; NS, not significant.

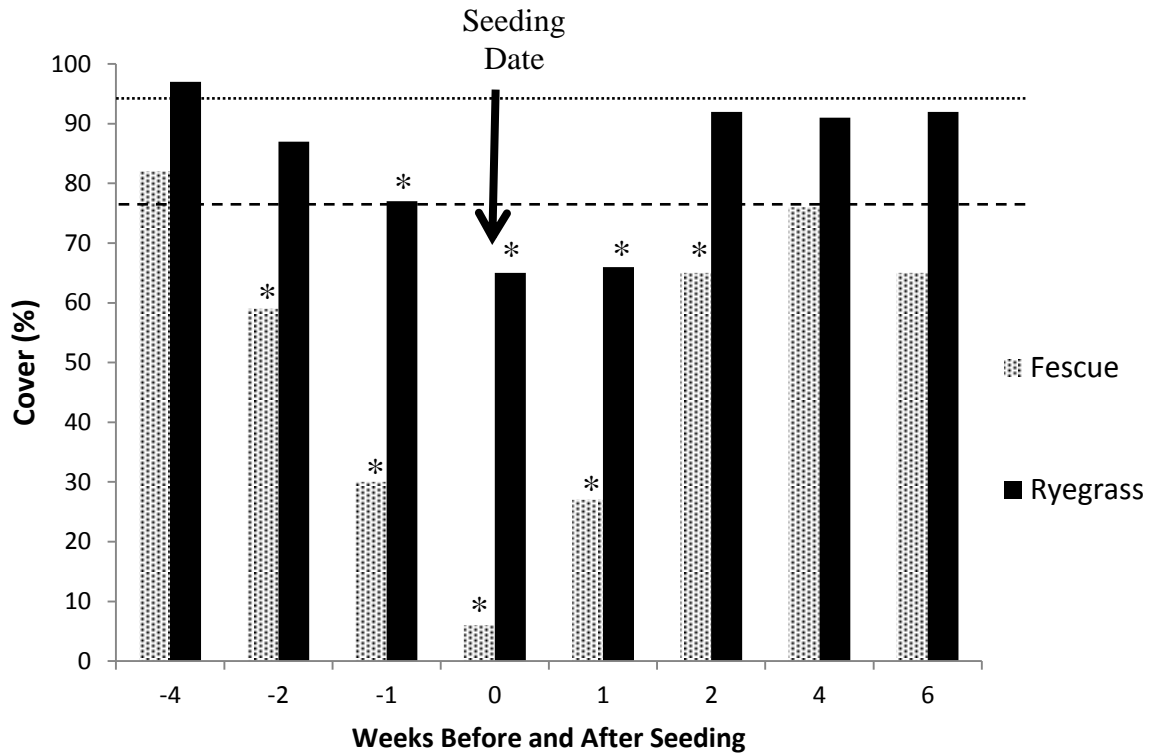


Figure 1. Effects of thaxtomin A at 570 g ai ha⁻¹ applied pre- and post-seeding on percent cover of newly seeded tall fescue and over-seeded perennial ryegrass. Data are from visual estimates of turfgrass cover evaluated 10 weeks after seeding. Dashed line represents average percent cover of tall fescue in non-treated plots. Dotted line represents average percent cover of perennial ryegrass in non-treated plots. The arrow notes the seeding date. Columns with an * were significantly different from the non-treated. Experiments conducted at the North Carolina Department of Agriculture and Consumer Services Sandhills Research Station (Jackson Springs, NC).

Table 6. Effect of applications of thaxtomin A on creeping bentgrass. Research conducted at the North Carolina State University Lake Wheeler Field Laboratory (Raleigh, NC).

Treatment	Dose at each application		Percent creeping bentgrass injury			
	1	2	4WAT ^a	8WAT	12WAT	16WAT
	-----g ai ha ⁻¹ -----		-----%-----			
Thaxtomin A	190	0	20	13	18	13
Thaxtomin A	380	0	38	28	43	33
Thaxtomin A	570	0	63	55	60	53
Thaxtomin A	190	190	20	40	40	38
Thaxtomin A	380	380	43	68	80	65
LSD _{0.05}			10	16	19	17

^a Abbreviations: WAT, weeks after seeding .

Part III

Postemergence Broadleaf Weed Control with the Bioherbicide FeHEDTA

Joseph C. Wolfe, Joseph C. Neal and Christopher D. Harlow*

Abstract. The worldwide demand and market for biopesticides has increased dramatically in recent years, but few natural products are available for weed control in turfgrass. FeHEDTA has recently been granted EPA registration as a bioherbicide, and is labelled for selective POST control of broadleaf weeds in turf. FeHEDTA efficacy on many economically important weeds has not been explored and recommendations on application volume, product dilution rates, and overall FeHEDTA dosing vary greatly. Field experiments were conducted to evaluate the impact of application parameters such as carrier volume, FeHEDTA concentration, FeHEDTA dose per unit area, and re-application intervals on broadleaf weed control. Additionally, field and container experiments were conducted to evaluate FeHEDTA efficacy on a diversity of regionally important broadleaf weeds. Common weeds evaluated in containers included dandelion, yellow woodsorrel, marsh yellowcress, ivyleaf speedwell, flexuous bittercress, henbit, and common chickweed. Weeds evaluated in newly-seeded tall fescue turf included dandelion, sparrow vetch, field madder, and common chickweed. Control of broadleaf weeds improved as FeHEDTA carrier volume and concentration increased. However, when compared across multiple carrier volumes, only FeHEDTA dose per unit area had a significant impact on weed control ($P < 0.0001$). In at least one year of container experiments, two applications of FeHEDTA provided control equal to that of an industry standard synthetic auxin herbicide for five of the seven weed species tested, but 30 to 52% control of henbit and less than 10% control of common chickweed. Results in newly-seeded turf were similar, with <30% control of common chickweed. In a separate experiment, three applications of FeHEDTA at two week intervals provided 100% control of henbit and 79-92% control of common chickweed. These data suggest that FeHEDTA dosing and re-application intervals are critical factors in determining its efficacy as an herbicide, and that FeHEDTA has the potential to provide commercially acceptable control of several weed species in areas where turf managers are unable or prefer not to utilize synthetic herbicides.

Nomenclature: FeHEDTA; 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; flexuous bittercress, *Cardamine flexuosa* With., CARFL; henbit, *Lamium amplexicaule* L., LAMAM; common chickweed, *Stellaria media* (L.) Vill., STEME; tall fescue, *Lolium arundinaceum* (Schreb.) S.J. Darbyshire ‘The Rebels’, ‘Top Choice’, FESAR; sparrow vetch, *Vicia tetrasperma* (L.) Schreb., VICTE; field madder, *Sherardia arvensis* L., SHRAR.

Key words: biological weed control; biopesticide; iron; dose response; application intervals.

* Graduate student, professor, and research technician, respectively, Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609. Corresponding author’s email: jcneal@ncsu.edu

Over the past decade the turfgrass industry has seen changes that present new challenges for turf managers attempting to control weeds. Public concerns over the risks to both humans and the environment posed by synthetic pesticide use have led to government agencies imposing regulations that restrict or ban the use of synthetic pesticides. In Canada, the provinces of Quebec and Ontario have either greatly restricted or banned the use of synthetic pesticides for aesthetic purposes in turf, while the state of New York's Child Safe Playing Fields Law has resulted in schools and daycare centers being banned from using synthetic chemical pesticides on lawns, athletic fields, and playgrounds (Belair et al. 2009; Grant 2011). These new restrictions have, in turn, generated greater interest in the development of new, organic or biologically-based weed control products (Boyetchko et al. 2009; Bailey et al. 2010).

The global pesticide market reflects these trends. Global spending on biopesticides increased as much as 10% annually from 2003 to 2005, with expenditures predicted to reach over US \$1 billion by 2010, owing largely to consumer safety concerns over synthetic pesticide use and the increasing popularity of organic and sustainable agriculture (Thakore 2006; Bailey et al. 2010). A 2004 survey also revealed that over 5 million homeowners in the United States use only organic lawn care products, with another 35 million using both synthetic and natural products (Anonymous 2004). Despite considerable interest in these products, however, biological herbicides have rarely been brought to market successfully (Ash 2010; Chandler et al. 2011).

In the United States, the only commercially available selective organic weed control option for use in turf is corn gluten meal, a byproduct of the corn milling process which has been shown to have provide PRE control of weedy summer annual grasses (Liu et al. 1994; McDade and Christians 2000). In more recent studies, corn gluten improved turfgrass quality but did not provide commercially acceptable weed control (Patton and Weisenberger 2011; St. John and DeMuro 2013; Siva 2014). An organic weed control option registered in Canada is *Sclerotinia minor*, a plant pathogen that has been evaluated as a potential biological herbicide for POST control of broadleaf weeds (Riddle et al. 1991; Schnick et al. 2002). As a live plant pathogen, the efficacy of *Sclerotinia minor* can vary greatly depending on factors such as moisture, temperature, and plant age, and its broad host range means that making applications near desirable dicotyledonous plants is not recommended (Abu-Dieyeh and Watson 2007; Bourdôt et al. 2011; Siva 2014). While these products offer some options for organic weed management, more tools are needed for managers to maintain commercially acceptable turf quality.

One alternative chemical control strategy currently being investigated is the use of chelated iron to control weed populations. Historically, both ferrous sulfate and iron chelates have been used for control of moss in certain turf situations (Burnell et al. 2004; Boesch and Mitkowski 2005). They are also widely used to mitigate the effects of iron deficiency in plants, and in some cases have been used to correct chlorosis caused by synthetic herbicides (Broschat 2003; Franzen et al. 2003; Chohura et al. 2009). Complexation of elemental iron into ferrous sulfate, ferrous citrate, or into forms such as FeDTPA, FeEDTA, or FeHEDTA using a chelating ligand improves absorption by target plants, as elemental iron is highly

insoluble (Broschat 2004; Hasegawa et al. 2011; Kolota et al. 2013). Synthetic chelating agents are currently the most popular method of iron complexation, as they provide the optimal combination of cost-efficiency and effectiveness (Hasegawa et al. 2011; Martins et al. 2014).

FeHEDTA (iron *N*-hydroxyethylethylenediaminetriacetate) is a chelated form of iron which has been approved by the United States Environmental Protection Agency for classification as a biopesticide, and has been labelled for use as a weed control agent in turf (Anonymous 2009). Early reports have indicated that POST applications of FeHEDTA can provide selective control of a large number of annual and perennial broadleaf weeds, causing foliar necrosis in target species within three days of application without injuring turf (Wilén 2012). While its mechanism of action and selectivity is not yet fully understood, it is possible that iron absorbed as FeHEDTA functions as a catalyst for oxygen reduction, leading to the formation of highly reactive oxygen species capable of causing cellular damage (Anonymous 2010).

Limited information is available on FeHEDTA's herbicidal activity. A series of trials conducted by Carey et al. (2010a, 2011a) revealed that two and three applications of FeHEDTA at 0.25, 0.5, and 1 g ai m⁻² applied at three week intervals in 1000, 2000 and 4000 L ha⁻¹ spray volumes provided control of broadleaf plantain (*Plantago major*), white clover (*Trifolium repens*), and common dandelion (*Taraxacum officinale*) equal to that of a synthetic auxin herbicide mixture. Siva (2014) reported that two applications of FeHEDTA at 1 g ai m⁻² applied in 4000 L ha⁻¹ spray volume reduced cover of broadleaf weeds such as common dandelion, white clover, and black medic (*Medicago lupulina*) to less than 5%. Lastly, Law et al. (2012) found that a single application of FeHEDTA failed to significantly reduce ground ivy coverage, though exact FeHEDTA dosing used in the experiment was not reported. These findings indicate that multiple applications of FeHEDTA may be necessary to significantly reduce weed populations, and that weed species may differ in susceptibility to FeHEDTA. Of the 14 broadleaf weed species reported in a 2012 survey to be common in turf in the southern United States (Webster 2012), FeHEDTA has only been tested on three. This illustrates the need for further research into the ability of FeHEDTA to control common weed species found in turfgrass.

Carey et al. (2010b, 2011b) also tested FeHEDTA for potential turfgrass injury, and demonstrated that up to eight applications of FeHEDTA at 1 g ai m⁻² applied every two weeks in a 4000 L ha⁻¹ spray volume did not significantly reduce red fescue (*Festuca rubra*), Kentucky bluegrass (*Poa pratensis*), or perennial ryegrass (*Lolium perenne*) quality. A further study conducted in 2011 on newly-seeded turf demonstrated that while FeHEDTA has the potential to injure these turf species when applied within one week of germination, applications made two to four weeks after germination resulted in significantly decreased weed populations and increased fine fescue and Kentucky bluegrass cover (Carey et al. 2011c).

Label guidelines for use of a commercially available formulation of FeHEDTA (26.52% FeHEDTA, 369.5 g ai L⁻¹) suggest a dilution of 39 mL product L⁻¹ applied at spray volumes

from 1020 to 4078 L ha⁻¹ with a follow-up application three to four weeks later (Anonymous 2013). These guidelines offer a large wide range of doses and costs per use which require further exploration. Past research into organic POST contact herbicides such as pelargonic acid, acetic acid, and pine oil has demonstrated that factors such as carrier volume, herbicide concentration, and application timing can have a significant impact on their performance (Johnson et al. 2004; Webber and Shefler 2007; Webber et al. 2014). However, research on other synthetic herbicides has demonstrated that the influence of these factors often varies depending upon herbicide formulation, mode of action, and the weed species in question (Harris 2010; Tuti and Das 2010; Doherty et al. 2011; Kieloch and Domaradzki 2011). The potential impact of such application variables on FeHEDTA efficacy has not been reported.

Thus far, research into FeHEDTA's herbicidal properties has been limited to a small number of weed species, and has not fully determined the application parameters required for commercially acceptable control of weeds exhibiting varying levels of susceptibility. The objectives of this research were to (1) determine the optimal combination of spray volume and FeHEDTA concentration for control of susceptible weeds, (2) determine the susceptibility of various regionally important weed species to FeHEDTA, and (3) determine FeHEDTA re-application intervals required to improve control of moderately susceptible weeds..

Materials and Methods

Field Sites and Experimental Design. Experiments were conducted at the North Carolina State University Lake Wheeler Field Laboratory in Raleigh, NC (35.74N, -78.88W) and at the North Carolina State University Horticultural Field Laboratory in Raleigh, NC (35.79N, -78.70W). The soil at the Lake Wheeler site was a Cecil sandy loam (fine, kaolinitic, thermic Typic Kanhapludults). The soil at the Horticultural Field Laboratory site was a Cecil gravelly sandy loam (fine, kaolinitic, thermic Typic Kanhapludults). The soil at both sites was highly disturbed by prior grading and construction activities. In each experiment, treated plots were 1 m² in size and treatments were arranged in randomized complete block designs with four replicates. Weed control was visually evaluated on a 0 to 10 scale where 0 = no control (no reduction in weed biomass relative to pre-treatment cover estimates) and 10 = 100% mortality of the weed. Intermediate values are visual estimates of percent reduction in above ground plant biomass. These data were converted to percent control for analysis and presentation. Additional measures of weed populations were recorded and are described below with each experiment.

Volume and Concentration Optimization. Volume and concentration optimization experiments were conducted at both the Lake Wheeler Field Laboratory and the Horticultural Field Laboratory sites. Test sites were selected for uniformly high weed populations. The Lake Wheeler site contained established stands of white clover (*Trifolium repens*) and dandelion (*Taraxacum officinale*) with little turfgrass cover. The Horticultural Field Laboratory site contained a mixed stand of winter annual broadleaf weeds including ivyleaf speedwell (*Veronica hederifolia*), Persian speedwell (*Veronica persica*), field madder (*Sherardia arvensis*), and common chickweed (*Stellaria media*). Trial areas received no

irrigation other than natural rainfall and were mowed weekly with a rotary mower at a height of 6.4 cm. Clippings were returned after each mowing.

The initial experiment was a factorial combination of three concentrations each applied at four spray volumes plus a non-treated control. The commercial formulation of FeHEDTA (Fiesta®, 26.52% FeHEDTA, 369.5 g ai L⁻¹; Neudorff, An der Muehle 3, Emmerthal, Germany 31860) was diluted 1.95%, 3.9% and 7.8% by volume in water and each dilution applied in 467, 935, 1870, and 3741 L ha⁻¹ spray volumes using a hand-held CO₂ pressurized sprayer equipped with two 8008 flat fan nozzles calibrated to deliver 467 L ha⁻¹. Higher spray volumes were achieved by multiple (2, 4, or 8) passes. The concentrations and spray volumes were selected based on the ranges of each recommended on the product label. Initial treatments were applied at both the Lake Wheeler and Horticultural Field Lab sites on October 16 and re-applied on November 6, 2012 when regrowth of weeds was observed. The experiment was repeated at the Lake Wheeler site in spring of 2014, with treatments applied on May 2 and re-applied on May 20, 2014.

Based on the results from the initial experiment, a second experiment was conducted in 2014 at the Lake Wheeler site to compare factorial combinations of four FeHEDTA doses each applied at 3 spray volumes. The doses tested, 6.75, 13.5, 27 and 54 kg ai ha⁻¹ FeHEDTA, were extrapolated from the prior concentration and spray volume experiment. Each dose was applied in 935, 1870, and 3741 L ha⁻¹ spray volumes. The equipment and application methods used in this trial were identical to those described above. Treatments were applied on April 17 and re-applied on May 9, 2014.

In addition to percent weed control evaluations conducted as described above, percent cover of white clover was also visually estimated. Weed control evaluations were recorded weekly until six weeks after the second application, at which time temperatures had halted weed growth. Final evaluations also included a 1 m² grid count to record white clover frequency. A 1 m² frame was divided into 100 equal sections of 1 dm² each. Sections containing living white clover were counted. Resulting counts provided a measure of percent frequency of living white clover.

Efficacy Screening (Containers). Experiments were conducted in containers at the Horticultural Field Laboratory site. Tests were initiated on September 12, 2012 and repeated on September 13, 2013. Common dandelion, yellow woodsorrel (*Oxalis stricta*), marsh yellowcress (*Rorippa islandica*), ivyleaf speedwell (*Veronica hederifolia*), flexuous bittercress (*Cardamine flexuosa*), henbit (*Lamium amplexicaule*), and common chickweed (*Stellaria media*) were surface seeded into 0.95-liter pots filled with a hammer-milled pine bark nursery substrate (Parker Bark Company, 3295 US Hwy 117, Rose Hill, NC 28458) amended with a controlled-release 18-4-8 (N-P₂O₅-K₂O) granular fertilizer (Harrell's Fertilizer Inc., 151 Gene E Stewart Blvd, Sylacauga, AL 35151) at a rate of 4.75 kg m⁻³. Pots were maintained in a covered shade house and overhead irrigated daily with about 1.5 cm of irrigation applied in 3 equal increments.

Treatments were applied about four weeks after seeding on October 16, 2012 and October 18, 2013 when weeds had reached an average height of two to five cm and grasses possessed

an average of three tillers. All treatments were re-applied three weeks later on November 6 in both 2012 and 2013. Treatments included a non-treated control, FeHEDTA at 29.6 and 59.1 kg ai ha⁻¹, and a ready-to-use auxin herbicide spray, Weed B Gon MAX RTU® (0.026% dicamba, 0.605% 2,4-dichlorophenoxyacetic acid, 0.149% methylchlorophenoxypropionic acid; The ORTHO Group, 1411 Scottslawn Road, Marysville, OH 43040), at 412 g ai ha⁻¹. Applications were made using a hand-held CO₂ pressurized sprayer equipped with two 8004 flat fan nozzles (TeeJet, Spraying Systems Co., North Ave. and Schmale Road, Wheaton, IL 60187) calibrated to deliver 280 L ha⁻¹.

The experiments were arranged in a randomized complete block design with five replications in 2012 and six replications in 2013 (a single pot of each weed species was included per treatment per replicate). Plant size was used as a blocking factor for replications. Weed control was visually evaluated weekly as described above.

POST Control of Broadleaf Weeds in Newly-Seeded Turf. Field experiments were initiated at the Horticultural Field Lab site 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 two weeks before seeding. Prior to seeding, debris was removed and the sites were raked smooth, core cultivated, and amended with a 9-13-7 (N-P₂O₅-K₂O) fertilizer (Ferti-lome New Lawn Starter Fertilizer®, Voluntary Purchasing Group, 230 FM 87, Bonham, Texas 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 Road NE, Salem, OR 97305). In both years, plots were seeded at a rate of 195 kg ha⁻¹ using a rotary spreader. After seeding, trial areas were irrigated as needed to promote establishment. Four weeks after seeding, turf received a second fertilization using the same fertilizer and dose as used prior to seeding. Following establishment, turf was mowed as needed at a 7.6 cm height with a rotary mower, with clippings returned to the site, and received supplemental irrigation as needed to prevent drought stress.

Treatments were applied on November 16, 2012 (7 weeks after seeding) and November 4, 2013 (eight weeks after seeding), respectively and included a non-treated control, FeHEDTA at 29.6 and 59.1 kg ai ha⁻¹, and Weed B Gon MAX RTU at 412 g ai ha⁻¹. In 2012, treatments were applied using a CO₂ pressurized backpack sprayer equipped with two 8008 flat fan nozzles calibrated to deliver 467 L ha⁻¹. In 2013, treatments were applied using the same CO₂-pressurized backpack sprayer equipped with two 8006 flat fan nozzles calibrated to deliver 280 L ha⁻¹.

In both 2012 and 2013, common dandelion, sparrow vetch (*Vicia tetrasperma*), large hop clover (*Trifolium campestre*), and common chickweed were the major weed species present at the time of application. Control of each of these species was evaluated visually one, two, and four weeks after application on a 0-10 scale as described above.

Application Intervals for Enhanced Efficacy on Henbit and Chickweed. Application interval experiments were initiated on October 25, 2013 and October 28, 2014 at the

Horticultural Field Lab site. Planting beds with heavy infestations of henbit and common chickweed were selected for the experiment.

Treatments in this experiment included FeHEDTA at rates of 29.6 and 59.1 kg ai ha⁻¹ applied three times at both two and three week intervals, along with a non-treated control. Treatments were applied with a hand-held CO₂ pressurized sprayer equipped with flat fan nozzles calibrated to deliver 280 L ha⁻¹. At the time of application the growth stage of both weed species ranged from cotyledons to 4 leaf pairs, at an average height of 5 cm.

Weed control in this experiment was evaluated weekly for 8 weeks using the 0 to 10 scale previously described. Additional evaluations were conducted 12 and 16 weeks after initial applications. At the conclusion of the trial henbit and common chickweed percent cover was estimated visually, and weed frequency distributions were documented by presence / absence counts using a 100 dm² grid as previously described.

Statistical Analysis. Data collected from all experiments were subjected to analysis of variance using Proc GLM in SAS (SAS 9.4, SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC 27513) to test for both main treatment effects and interactions. Where treatment effects were determined to be significant, treatment means were separated using Fisher's protected least significant difference 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.

Data from volume, concentration and dosing factorial experiments were further subjected to nonlinear regression analysis over FeHEDTA dose using the four-parameter log-logistic model described in Equation 1 (Price et al. 2012):

$$Y = C + (D - C) / \{1 + \exp[B(\log X - \log E)]\} \quad [1]$$

Where Y is the response (percent control), C is the lower limit, D is the upper limit, B is the slope of the line at the inflection point, X is the FeHEDTA dose, and E is the dose resulting in a 50% response between C and D (also known as the inflection point, I_{50}). Because a significant treatment-by-experiment interaction was detected, data from each experiment are presented separately. An F test for lack of fit at the 5% level was not significant for any of the dose response curves tested, indicating that the log-logistic model was appropriate (Price et al. 2012).

Results and Discussion

Volume and Concentration Optimization. Treatment-by-species interactions were significant at all rating dates, thus data from each species is presented separately. Treatment-by-year interactions were significant for percent white clover control after two applications and percent white clover cover after a single application, and thus data from these years are presented separately.

Three weeks after the first application of FeHEDTA, percent control of white clover increased from a low of 20% to a high of 80% as both carrier volume and FeHEDTA

concentration increased (Table 1). A similar decrease in percent cover of white clover was observed as spray volume and concentration were increased. The increase in percent control was negatively correlated with the decrease in percent cover ($R = -0.99$). Analysis of variance revealed that main effects of both spray volume and concentration were significant, but the interaction between these factors was not (Table 1). Following a second application, white clover control increased and percent cover decreased for most combinations of volume and concentration.

Similar results were observed on winter annual broadleaf weeds in a separate experiment. Percent control of winter annual broadleaf weeds increased as volume and FeHEDTA concentrations increased. Percent control ranged from 22% to 77% at the 1.95% concentration, from 35% to 85% at the 3.8% concentration, and from 77% to 96% at the 7.8% concentration, respectively (Table 2). Both main effects were significant after one and two applications. In this experiment, the volume-by-concentration interaction was significant following the second application. At all combinations of volume and concentration, percent control of winter annual broadleaf weeds increased following a second application of FeHEDTA (Table 2).

In 2012, percent control of dandelion with one application of FeHEDTA improved as volume and concentration increased. Both main effects of volume and concentration were significant, but the interaction of volume-by-concentration was not significant (Table 2). Following a second application of FeHEDTA, percent control of dandelion increased at all combinations of volume and concentration, though only the main effect of volume was significant. In contrast, all treatments provided 100% control of dandelion in the 2014 experiment, thus no main effects were significant. Differences in results between years may have resulted from significantly lower dandelion populations in 2014 compared to 2012. In 2012, dandelion populations averaged 10 dandelions per m^2 , compared to 2 dandelions per m^2 in 2014.

These results indicate that weed species vary in their susceptibility to FeHEDTA. The differences in efficacy between years observed on both white clover and dandelion implies that application timing, population size, and plant maturity may also have a role in determining FeHEDTA efficacy.

When data were analyzed for responses based on the total dose of FeHEDTA per unit area, no differences between spray volumes or concentrations were observed (Table 1). When these data were plotted, the resulting means fit a non-linear dose response curve (Figure 1). However, because these experiments resulted in an unbalanced data set for dose response analysis, a final experiment was conducted in which four doses were each applied at three spray volumes.

Data from the dose and spray volume factorial study confirmed that, regardless of spray volume, white clover control increased with increasing dose of FeHEDTA. When these data were plotted, the resulting means fit a non-linear dose response curve (Figure 2). Analysis of variance indicated that dose was highly significant for both white clover control and cover, while volume and dose-by-volume interaction are not significant (Table 3). Regardless of spray volume, white clover was controlled $\geq 87\%$ to $\geq 93\%$ when FeHEDTA was applied at

27 kg ai ha⁻¹ or 54 kg ai ha⁻¹ respectively (Table 3). These results differ from those reported by Carey et al. (2010a, 2011a), who reported that two applications of FeHEDTA at 10 kg ai ha⁻¹ provided nearly complete control of white clover within four weeks of the second application. Differences between these two experiments may be due to the differences in population size between the two experiments, as percent white clover cover in Carey's experiments averaged only 16-17% compared to 75% in this experiment. Weed maturity at the time of application may have also been a factor. The presence of dense, healthy turf in Carey et al.'s experiments may have also contributed to the superior control that was observed, as increased turf competition has been associated with reductions in weed populations (Elfort et al. 2008). Climatic differences in the two regions may have also influenced these results, though the absence of environmental data in Carey et al.'s reports precludes the possibility of direct comparisons.

Efficacy Screening (Containers). Treatment-by-year interaction was significant for henbit, birdeye pearlwort, and flexuous bittercress, and not significant for other species tested. Thus, data for these three species are presented separately by year, while data for the other species were pooled across years.

Two applications of FeHEDTA at 29.7 and 59.4 kg ai ha⁻¹ controlled seedling dandelion 97 and 100%, respectively, equivalent to the auxinic herbicide (Table 5). At 29.7 kg ai ha⁻¹ FeHEDTA also provided 93% control of yellow woodsorrel, equivalent to the auxinic herbicide. FeHEDTA at 59.4 kg ai ha⁻¹ provided 99% control which was significantly better control than that of the auxinic herbicide. Marsh yellowcress was controlled 94% by FeHEDTA at 59.4 kg ai ha⁻¹, but control at 29.7 kg ai ha⁻¹ was significantly lower, 86%, and was not equivalent to the auxinic herbicide. Control of ivyleaf speedwell was >90% at both rates of FeHEDTA; whereas the auxinic herbicide provided less than 20% control of this species. Common chickweed was controlled <10% by both rates of FeHEDTA, but was controlled 99% by the auxinic herbicide.

In 2012, henbit was controlled <40% by both doses of FeHEDTA, but was still equivalent to the auxinic herbicide which provided only 24% control. In 2013, henbit was controlled 30% and 52% by 29.7 and 59.4 kg ai ha⁻¹ FeHEDTA, respectively. Henbit control with the auxinic herbicide was significantly better at 82% (Table 5). Flexuous bittercress was controlled 28% and 50% by FeHEDTA at 29.7 and 59.4 kg ai ha⁻¹ in 2012 but improved to 56% and 93% in 2013. In 2013, FeHEDTA at 59.4 kg ai ha⁻¹ provided control of flexuous bittercress equivalent to that of the auxinic herbicide (Table 5).

It is possible that these differences between years resulted from variation in temperatures. At the time of the second application, the ambient temperature was 10.6 degrees C greater in 2013 than 2012 (6.7 and 17.2 in 2012 and 2013, respectively). Blackshaw et al. (2002) reported that henbit emergence and growth was greatest at temperatures between 15 and 20 degrees C, closer to the temperatures at the time of application in 2013. Thus, weather conditions in 2013 may have favored growth of henbit and flexuous bittercress, while colder temperatures observed in 2012 may have slowed growth rates and thus reduced sensitivity to FeHEDTA. Similarly, greater efficacy of the auxinic herbicide in 2013 aligns with past

reports that plant sensitivity to synthetic auxin herbicides like 2,4-D and dicamba increases at higher temperatures (Al-Khatib et al. 1999; Kittock & Arle 1977).

POST Control of Broadleaf Weeds in Newly-Seeded Turf. Within two days of application FeHEDTA had caused nearly complete necrosis of above-ground plant tissue of dandelion, while the industry standard auxinic herbicide had not caused injury (Table 6). Within 4 weeks of application both doses of FeHEDTA provided 99% control of dandelion, superior to that of the auxinic herbicide, which provided only 50% control. Control of large hop clover with FeHEDTA was $\geq 93\%$ at both doses, and was superior to the auxinic herbicide. All treatments provided similar control of sparrow vetch, 79-89%. Common chickweed control was $< 30\%$ with both rates of FeHEDTA, while the auxinic herbicide provided 89% control (Table 6). Control of dandelion and chickweed in this experiment was similar to that observed in the container experiment described above. Based on these findings, it is likely that the efficacy of FeHEDTA on other weed species tested in containers provides a realistic estimate of what will be observed when FeHEDTA is applied to the same species in turf.

These data suggest that while FeHEDTA can provide rapid and effective control of a number of broadleaf weed species in turf such as dandelion, sparrow vetch and large hop clover, the presence of some less-sensitive species such as common chickweed may result in poor overall weed control compared to an industry standard synthetic auxin herbicide.

Application Intervals for Enhanced Efficacy on Henbit and Chickweed. Year-by-treatment interactions were significant for both species in this experiment, and thus data from 2013 and 2014 are presented separately. In 2013, dose and application interval were significant for control of both henbit and common chickweed, but no interaction was detected (Table 7). Control of both weeds was improved by increasing FeHEDTA dose and by reducing the re-application interval from every three weeks to every two weeks. Percent cover of henbit and common chickweed was reduced by increasing FeHEDTA dose from 29.7 kg ai ha⁻¹ to 59.4 kg ai ha⁻¹, but on henbit no difference between 2-week and 3-week application intervals was detected. Percent control and percent cover of henbit and chickweed had negative correlations ($R = -0.998$ for henbit, $R = -0.812$ for chickweed). FeHEDTA at 59.4 kg ai ha⁻¹ provided 99-100% control of henbit regardless of application interval. Two-week application intervals provided superior control of henbit than three week application intervals when FeHEDTA was applied at 29.7 kg ai ha⁻¹ (91% and 81% control, respectively), though no significant differences were seen in percent henbit cover at the conclusion of the experiment. For common chickweed, application dose, treatment interval and the interaction were significant. Common chickweed was also controlled similarly by FeHEDTA at 59.4 kg ai ha⁻¹ regardless of application intervals. However, when applied at 29.7 kg ai ha⁻¹, two-week application intervals provided better control of common chickweed than three week intervals (40% and 2% control, respectively). At 29.7 kg ai ha⁻¹, FeHEDTA did not reduce common chickweed cover at either application interval, and actually resulted in increased common chickweed cover compared to the non-treated when applied at 3 week intervals (Table 7). This was most likely due to the reduction in henbit cover leading to less competition for common chickweed after FeHEDTA was applied.

In 2014 no differences in henbit control were observed between any FeHEDTA treated plots, as all rates of FeHEDTA provided 100% control. Control of common chickweed with FeHEDTA at 59.4 kg ai ha⁻¹ was improved when applied at two-week application intervals compared to three-week application intervals, 79% and 51% control, respectively (Table 7). In 2014, common chickweed was not controlled by FeHEDTA at 29.7 kg ai ha⁻¹, regardless of application interval. Much like in 2013, both main effects of dose and application interval were significant for common chickweed, but no interaction was detected.

The differences observed between the two years is likely due to differences in the henbit and chickweed density. In 2013, henbit was the predominant species in all plots with essentially 100% cover in all plots when the experiment was initiated. Common chickweed seedlings were present in smaller populations, and germinated later than the henbit. In 2014 common chickweed was the more prevalent species with about 67% cover in the non-treated plots compared to 33% henbit cover. The smaller size of the henbit population in 2014 may have made FeHEDTA injury more pronounced, while the established population of common chickweed may have resulted in less control with FeHEDTA compared to 2013.

These data demonstrate that it is possible to improve the control of some more difficult to control species by increasing FeHEDTA application dose and frequency. In both years, control of henbit following three applications of FeHEDTA was substantially better than the control observed after one and two applications in prior field studies and container screenings. Control of common chickweed likewise improved, though efficacy varied between years and may have been impacted by population size and growth stage at the time of application. This improvement in control following multiple applications is similar to the results reported for pelargonic acid. Webber et al. (2014) observed a 21% increase in broadleaf weed control, a 30% increase in smooth crabgrass (*Digitaria ischaemum*) control, and a 33% increase in yellow nutsedge (*Cyperus esculentus*) control immediately following a second application of pelargonic acid.

The results of these experiments demonstrate that carrier volume and concentration have little or no impact on FeHEDTA efficacy, provided a sufficient dose per unit area is applied. Furthermore, these results suggest that FeHEDTA can provide commercially acceptable control of several economically important broadleaf weed species, though species response is variable and some species, such as henbit and common chickweed, are less susceptible to control with FeHEDTA. Control of these species can be substantially improved by reducing the interval between applications to two weeks. While maintaining commercially acceptable turfgrass using only biological products remains a challenge for turf managers, FeHEDTA may provide an additional tool to aid in accomplishing this goal.

Acknowledgements

The authors express appreciation to the USDA IR-4 Biopesticides Program and Neudorff Co. for funding this research.

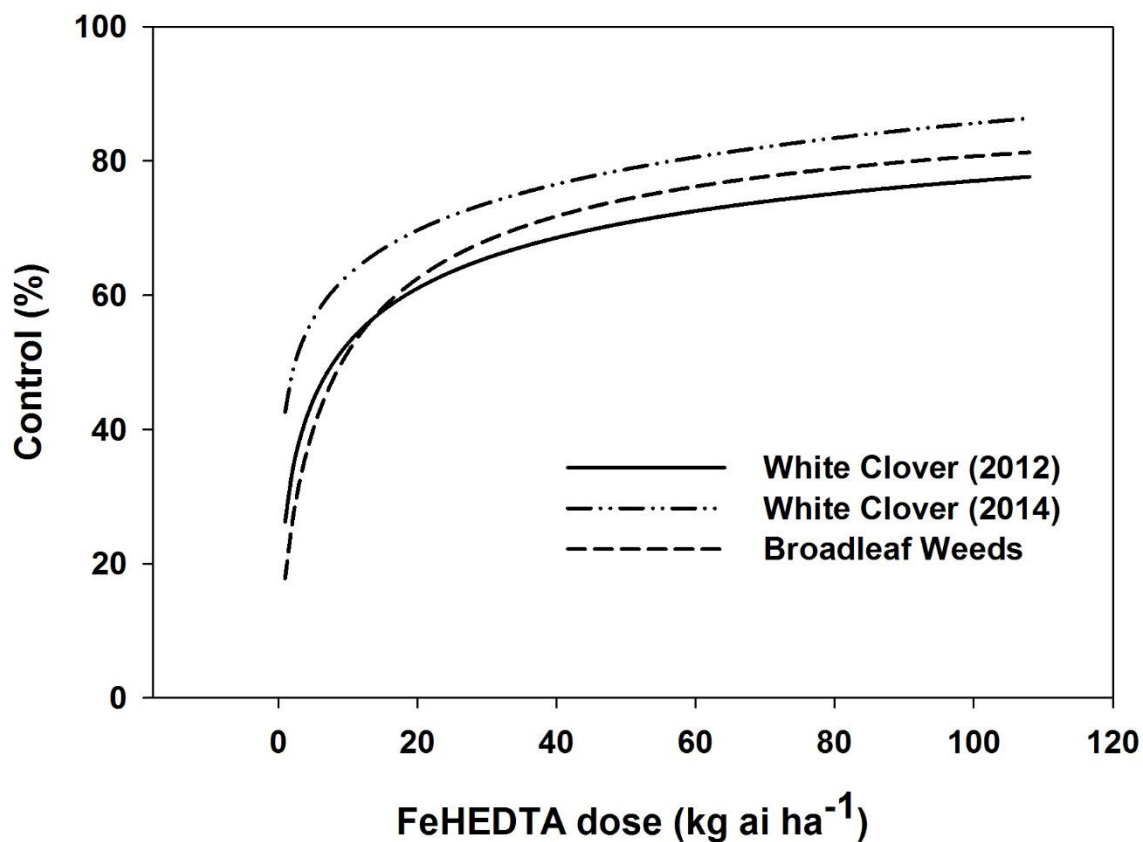


Figure 1. Effect of FeHEDTA dose on control (%) of white clover and winter annual broadleaf weeds; evaluated six weeks after second application on white clover and four weeks after second application on winter annual broadleaf weeds (6WAT2 and 4WAT2, respectively). The regression lines were plotted using Equation 1, and the parameter values are presented in Table 4.

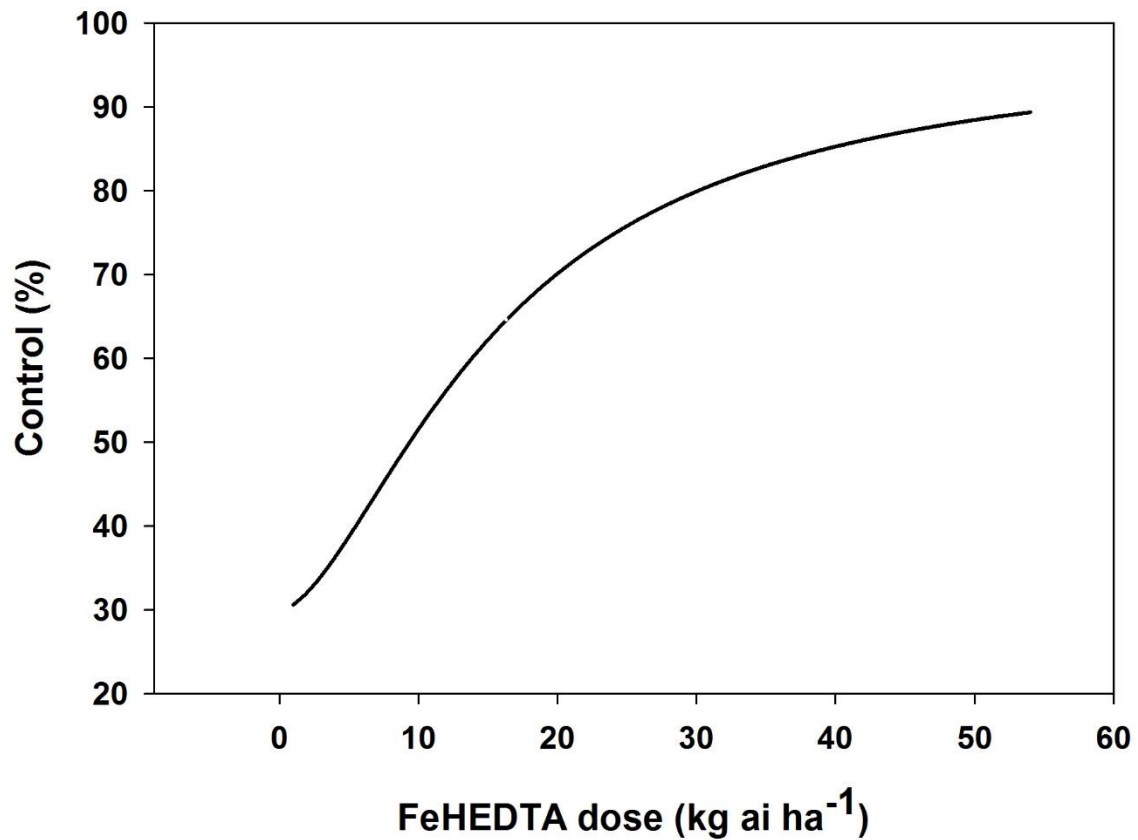


Figure 2. Effect of FeHEDTA dose on control (%) of white clover, six weeks after second application (6WAT2). The regression line was plotted using Equation 1, and the parameter values are presented in Table 4.

Table 1. Percent control and percent cover of white clover following one and two applications of FeHEDTA at multiple combinations of concentration and spray volume. Research conducted at the North Carolina State University Lake Wheeler Field Laboratory (Raleigh, NC).

Concentration (v/v)	Volume (L ha ⁻¹)	Dose (kg ai ha ⁻¹)	3WAT1 ^{ab} -----% Control ^c -----	6WAT2		3WAT1		6WAT2
				2012	2014	2012	2014	
1.95	467	3.375	20	22	32	51	55	59
1.95	935	6.75	26	50	35	36	52	55
1.95	1870	13.5	50	69	50	19	36	34
1.95	3741	27	57	70	71	22	31	26
3.9	467	6.75	42	52	45	26	46	43
3.9	935	13.5	47	67	40	34	55	39
3.9	1870	27	59	80	70	15	40	21
3.9	3741	54	75	87	79	16	25	14
7.8	467	13.5	45	65	50	27	55	39
7.8	935	27	66	84	71	20	36	21
7.8	1870	54	71	91	87	17	24	10
7.8	3741	108	82	98	96	7	21	3
LSD _{0.05}			14	16	13	19	21	13
ANOVA P values								
Volume			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Concentration			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Volume X Concentration			---NS---	---NS---	---NS---	---NS---	---NS---	---NS---

^a Abbreviations: 3WAT1, 3 weeks after the first treatment; 6WAT2, 6 weeks after the second treatment; NS, not significant.

^b Where the interaction between year and treatment effect is not significant, data were pooled across years. Where a significant interaction between years were detected, data are presented by year.

^c Percent control based on visual estimations of reductions in above ground plant biomass, where 0 = no reduction and 100 = complete plant mortality.

Table 2. Percent control of mixed winter annual broadleaf weeds and dandelion following one and two applications of FeHEDTA at multiple combinations of concentration and spray volume. Research conducted at the North Carolina State University Lake Wheeler Field Laboratory and Horticultural Field Laboratory (Raleigh, NC).

Concentration	Volume	Dose	WABRDLF ^{ab}		TAROF ^c			
			3WAT1	4WAT2	3WAT1		6WAT2	
(v/v)	(L ha ⁻¹)	(kg ai ha ⁻¹)	-----% Control ^d -----					
1.95	467	3.375	13	22	35	100	45	100
1.95	935	6.75	27	43	50	100	52	100
1.95	1870	13.5	30	67	60	100	57	100
1.95	3741	27	55	77	62	100	67	100
3.9	467	6.75	28	35	42	100	35	100
3.9	935	13.5	48	60	57	100	52	100
3.9	1870	27	60	80	67	100	80	100
3.9	3741	54	63	85	82	100	87	100
7.8	467	13.5	53	77	57	100	47	100
7.8	935	27	50	82	57	100	47	100
7.8	1870	54	75	95	87	100	90	100
7.8	3741	108	80	96	94	100	99	100
LSD _{0.05}			25	17	20	--NS--	33	--NS--
ANOVA P values								
Volume			0.0001	<0.0001	<0.0001	--NS--	<0.0001	--NS--
Concentration			<0.0001	<0.0001	0.0057	--NS--	---NS---	--NS--
Volume X Concentration			---NS---	0.0331	---NS---	--NS--	---NS---	--NS--

^a Abbreviations: WABRDLF, winter annual broadleaf weeds; TAROF, dandelion; 3WAT1, 3 weeks after treatment 1; 4WAT2, 4 weeks after treatment 2; 6WAT2, 6 weeks after treatment 2; NS, not significant.

^b Mixed winter annual broadleaf weeds includes Persian speedwell, ivyleaf speedwell, common chickweed, and field madder.

^c Data presented by year as year-by-treatment effect was significant.

^d Percent control based on visual estimations of reductions in above ground plant biomass, where 0 = no reduction and 100 =

complete plant mortality.

Table 3. Percent control and percent cover of white clover after one and two applications of FeHEDTA at multiple doses and spray volumes. Research conducted at the North Carolina State University Lake Wheeler Field Laboratory (Raleigh, NC).

Dose	Volume	3WAT1 ^a	6WAT2	3WAT1	6WAT2
(kg ai ha ⁻¹)	(L ha ⁻¹)	-----% Control ^b -----		-----% Cover-----	
6.75	935	13	35	74	35
6.75	1870	10	30	65	42
6.75	3741	13	32	67	40
13.5	935	18	55	62	25
13.5	1870	23	52	52	27
13.5	3741	25	55	45	25
27	935	25	94	52	6
27	1870	33	87	45	12
27	3741	28	87	57	14
54	935	35	96	39	3
54	1870	45	100	34	0
54	3741	40	93	40	6
LSD _{0.05}		11	9	16	15
ANOVA P values					
Dose		<0.0001	<0.0001	<0.0001	<0.0001
Volume		NS	NS	NS	NS
Dose X Volume		NS	NS	NS	NS

^a Abbreviations: 3WAT1, 3 weeks after the first treatment ; 6WAT2, 6 weeks after the second treatment ; NS, not significant.

^b Percent control based on visual estimations of reductions in above ground plant biomass, where 0 = no reduction and 100 = complete plant mortality.

Table 4. Model parameters and standard errors for the four-parameter log-logistic model (provided in Equation 1) for Figures 1 and 2 for white clover and winter annual broadleaf weed response to two POST applications of FeHEDTA.

Experiment	Model Parameters (\pm SE)			
	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>
Conc. Factorial (2012)	1.14 (0.23)	-1.123 (5)	98 (6)	7 (1.16)
Conc. Factorial (2014)	0.5966 (0.19)	0.661 (5)	154 (60)	43.2 (57)
Broadleaf Weeds (2012)	1.67 (0.29)	0.367 (5)	93 (4)	7.5 (0.85)
Dosing Factorial (2014)	3.8 (0.66)	29.9 (3)	97.1 (2)	15.7 (0.68)

Table 5. Percent control of container-grown broadleaf weeds following two applications of FeHEDTA and Weed B Gon. Research conducted in outdoor containers at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

Herbicide	Dose kg ai ha ⁻¹	TAROF ^{ab}	OXAST	RORIS	VERHE	STEME	LAMAM ^c		CARFL	
							2012	2013	2012	2013
		-----% Control ^d -----								
FeHEDTA	29.7	100	93	86	93	7	36	30	28	56
FeHEDTA	59.4	97	99	94	96	8	38	52	50	93
Weed B Gon ^e	0.412	94	95	100	79	99	24	82	100	100
LSD _{0.05}		6	4	7	9	8	21	26	24	30

^a Abbreviations: TAROF, dandelion; OXAST, yellow woodsorrel; RORIS, marsh yellowcress; VERHE, ivyleaf speedwell; STEME, common chickweed; LAMAM, henbit; CARFL, flexuous bittercress.

^b Data collected 6 weeks after second application.

^c Where the interaction between year and treatment effect is not significant, data were pooled across years. Where a significant interaction between years were detected, data are presented by year.

^d Percent control based on visual estimations of reductions in above ground plant biomass, where 0 = no reduction and 100 = complete plant mortality.

^e 0.026% dicamba, 0.605% 2,4-dichlorophenoxyacetic acid, 0.149% methylchlorophenoxypropionic acid

Table 6. Percent necrosis of dandelion in tall fescue turf two days after applications of FeHEDTA and Weed B Gon and control of broadleaf weeds in tall fescue turf four weeks after applications of FeHEDTA and Weed B Gon. Research conducted at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

Herbicide	Dose kg ai ha ⁻¹	TAROF ^{ab}		VICTE	TRFCA	STEME
		2DAT	4WAT			
		-----% Control ^c -----				
FeHEDTA	29.7	90	99	79	93	16
FeHEDTA	59.4	95	99	89	96	30
Weed B Gon ^d	0.412	1	50	85	79	89
LSD _{0.05}		5	13	10	24	18

^a Abbreviations: TAROF, dandelion; VICTE, sparrow vetch; TRFCA, large hop clover; STEME, common chickweed; 2DAT, 2 days after treatment; 4WAT, 4 weeks after treatment.

^b Data pooled across years as year-by-treatment interaction was not significant.

^c Percent control based on visual estimations of reductions in above ground plant biomass, where 0 = no reduction and 100 = complete plant mortality.

^d 0.026% dicamba, 0.605% 2,4-dichlorophenoxyacetic acid, 0.149% methylchlorophenoxypropionic acid

Table 7. Percent control and percent cover of henbit and common chickweed following 3 applications of FeHEDTA at multiple doses and application intervals. Research conducted at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

Dose	Interval	LAMAM ^{ab}		STEME		LAMAM		STEME	
		2013	2014	2013	2014	2013	2014	2013	2014
kg ai ha ⁻¹		-----% Control ^c -----				-----% Cover-----			
0	NA	--	--	--	--	77	17	16	67
29.7	2WK	91	100	40	5	6	0	8	70
29.7	3WK	81	100	2	0	12	0	32	82
59.4	2WK	100	100	92	79	0	0	1	18
59.4	3WK	99	100	76	53	0	0	3	31
LSD _{0.05}		8	-NS-	25	17	9	4	13	21
ANOVA P values									
Dose		0.0006	NS	<0.0001	<0.0001	0.0148	NS	0.0038	0.0002
Interval		0.0455	NS	0.0208	0.0332	NS	NS	0.0241	NS
Dose X Interval		NS	NS	NS	NS	NS	NS	0.05	NS

^a Abbreviations: LAMAM, henbit; STEME, common chickweed; NA, not applicable; WK, week.

^b Data presented by year as year-by-treatment effect was significant.

^c Percent control based on visual estimations of reductions in above ground plant biomass, where 0 = no reduction and 100 = complete plant mortality.

Literature Cited

- Abu-Dieyeh MH, Watson AK (2007) Efficacy of *Sclerotinia minor* for dandelion control: effect of dandelion accession, age, and grass competition. *Weed Res* 47: 63-72
- Al-Khatib K, Peterson D (1999) Soybean (*Glycine max*) response to simulated drift from selected sulfonylurea herbicides, dicamba, glyphosate, and glufosinate. *Weed Technol* 13: 264-270
- Anonymous (2004) Environmental Lawn and Garden Survey. *Organic Gardening Magazine*. http://www.gro-well.com/files/5613/6511/4280/2004_Environmental_Lawn_Garden_Survey.pdf. Accessed: July 18, 2014. 90p
- Anonymous (2009) Proposed Registration Action Document: Iron HEDTA. United States Environmental Protection Agency. http://www.epa.gov/pesticides/chem_search/reg_actions/registration/decision_PC-034702_17-Sep-09.pdf. Accessed: July 10, 2014. 16p
- Anonymous (2010) Proposed Registration Decision: FeHEDTA. Health Canada. http://publications.gc.ca/site/archive-archived.html?url=http://publications.gc.ca/collections/collection_2011sc-hc/H113-25-2010-9-eng.pdf. Accessed: July 18, 2014. 19p
- Anonymous (2013) Fiesta® herbicide product label. Engage Agro USA Publication. Prescott, AZ: Engage Agro USA. 6p
- Ash GJ (2010) The science, art and business of successful bioherbicides. *Biol Control* 52(3):230-40
- Bailey KL, Boyetchko SM, Längle T (2010) Social and economic drivers shaping the future of biological control: A Canadian perspective on the factors affecting the development and use of microbial biopesticides. *Biol Control* 52(3):221-9
- Bélaïr G, Koppenhöfer AM, Dionne J, Simard L (2010) Current and potential use of pathogens in the management of turfgrass insects as affected by new pesticide regulations in North America. *Int J Pest Manage* 56(1):51-60
- Blackshaw RE, Brandt RN, Entz T (2002) Soil temperature and soil water effects on henbit emergence. *Weed Sci* 50(4):494-497
- Boesch BP, Mitkowski NA (2005) Chemical methods of moss control on golf course putting greens. *Appl Turfgrass Sci* DOI:10.1094/ATS-2005-1006-01-RV
- Bourdôt GW, Saville DJ, Jong MD (2011) Evaluating the environmental safety of broad-host-range bioherbicides. *Pest Technol* 5:34-40
- Boyetchko SM, Bailey KL, De Clerck-Floate RA (2009) Current biological weed control agents - their adoption and future prospects. *Prairie Soils and Crops* 2:6
- Broschat TK (2003) Effectiveness of various iron sources for correcting iron chlorosis in dwarf ixora. *HortTechnology* 13(4):625-627
- Broschat TK, Moore KK (2004) Phytotoxicity of several iron fertilizers and their effects on Fe, Mn, Zn, Cu and P content of African marigolds and zonal geraniums. *HortScience* 39(3): 595-598

- Burnell KD, Yelverton FH, Neal JC, Gannon TW, McElroy JS (2004) Control of silvery-thread moss (*bryum argenteum hedw.*) in creeping bentgrass (*agrostis palustris huds.*) putting greens. *Weed Technol* 18(3):560-565
- Carey K, Porter AJ, Lyons EM, Jordan KS (2010a) Herbicidal activity of NEU1173H applied to turfgrass infested with dandelion, broadleaved plantain, and white clover – Spring 2010 trial. Guelph, ON: Department of Plant Agriculture and Guelph Turfgrass Institute Annual Research Report, University of Guelph, Ontario.
http://www.guelph turfgrass.ca/sites/default/files/research_reports/2010-Herbicidal%20activity%20of%20NEU1173H%20applied%20to%20turfgrass%20infested%20with%20dandelion%2C%20broadleaved%20plantain%2C%20and%20white%20clover.pdf. Accessed: April 6, 2015. 5p
- Carey K, Porter AJ, Lyons EM, Jordan KS (2010b) Tolerance of different grass species to repeated applications of NEU1173H – Spring 2010 trial. Guelph, ON: Department of Plant Agriculture and Guelph Turfgrass Institute, University of Guelph, Ontario.
http://www.guelph turfgrass.ca/sites/default/files/research_reports/2010-Tolerance%20of%20different%20grass%20species%20to%20repeated%20application%20of%20NEU1173H.pdf. Accessed: April 6, 2015. 9p
- Carey K, Porter AJ, Lyons EM, Jordan KS (2011a) Herbicidal activity of NEU1173H applied to turfgrass infested with dandelion, broadleaved plantain, and white clover – Spring 2011 trial. Guelph, ON: Department of Plant Agriculture and Guelph Turfgrass Institute, University of Guelph, Ontario.
http://www.guelph turfgrass.ca/sites/default/files/research_reports/2012-Herbicidal%20activity%20of%20NEU1173H%20applied%20to%20turfgrass%20infested%20with%20dandelion%2C%20broadleaved%20plantain%2C%20and%20white%20clover%20-%20Spring%202011%20trial.pdf. Accessed: April 6, 2015. 4p
- Carey K, Porter AJ, Lyons EM, Jordan KS (2011b) Tolerance of different grass species to repeated applications of NEU1173H – Spring 2011 trial. Guelph, ON: Department of Plant Agriculture and Guelph Turfgrass Institute, University of Guelph, Ontario.
http://www.guelph turfgrass.ca/sites/default/files/research_reports/2012-Tolerance%20of%20different%20grass%20species%20to%20repeated%20application%20of%20NEU1173H%20-%202011%20trial.pdf. Accessed: April 6, 2015. 10p
- Carey K, Porter AJ, Lyons EM, Jordan KS (2011c) Tolerance of new seedings of different grass species to application of NEU1173H and efficacy of NEU1173H for weed control in newly seeded turf - 2011. Guelph, ON: Department of Plant Agriculture and Guelph Turfgrass Institute, University of Guelph, Ontario.
http://www.guelph turfgrass.ca/sites/default/files/research_reports/2012-Tolerance%20of%20new%20seedings%20of%20different%20grass%20species%20to%20application%20of%20NEU1173H%20and%20efficacy%20of%20NEU1173H%20for%20weed%20control%20in%20newly%20seeded%20turf%20-%202011.pdf. Accessed: May 21, 2015. 10p
- Chandler D, Bailey AS, Tatchell GM, Davidson G, Greaves J, Grant WP (2011) The development, regulation and use of biopesticides for integrated pest management. *Philos Trans R Soc, B* 366(1573):1987-98

- Chohura P, Kolota E, Komosa A (2009) Effect of fertilization with Fe chelates on the state of iron nutrition in greenhouse tomato. *J Elem* 14(4): 657-664
- Doherty RC, Smith KL, Bullington JA, Meier JR (2011) Palmer amaranth and barnyardgrass control as influenced by weed size, glufosinate rate, volume, and spray tip. Monticello, AK: University of Arkansas AAES Research Series (589): 115-118
- Elfort EMA, Tardif FJ, Robinson DE, Lyons EM (2008) Effect of perennial ryegrass overseeding on weed suppression and sward composition. *Weed Technol* 22:231-23
- Franzen DW, O'Barr JH, Zollinger RK (2003) Interaction of a foliar application of iron HEDTA and three postemergence broadleaf herbicides with soybeans stressed from chlorosis. *J Plant Nutr* 26(12):2365-2374
- Grant J (2011) The child safe playing fields act: NY's ban on pesticide use on school and day care center grounds. Ithaca, NY: Cornell University NYS IPM Program
- Johnson E, Wolf T, Caldwell B, Barbour R, Holm R, Sapsford K (2004) Efficacy of vinegar (acetic acid) as an organic herbicide. [http://www.spokane-county.wsu.edu/smallfarms/organic %20ag/ADF%20Vinegar%20Report%20Final.pdf](http://www.spokane-county.wsu.edu/smallfarms/organic%20ag/ADF%20Vinegar%20Report%20Final.pdf). Accessed July 23, 2014
- Harris D (2010) The influence of spray application volume for volunteer potato control. Pages 161-164 *in* Proceedings of Crop Protection in Northern Britain. Dundee, UK: The Dundee Conference
- Hasegawa H, Azizur Raman M, Saitou K, Kobayashi M, Okumura C (2011) Influence of chelating ligands on bioavailability and mobility of iron in plant growth media and their effect on radish growth. *Eviron Exp Bot* 71(3): 345-351
- Hephner AJ, Cooper T, Beck LL, Henry GM (2012) Sequential postemergence applications for the control of khakiweed in bermudagrass turf. *HortScience* 47(3): 434-436
- Karim Dagno RL, Diourté M, Jijakli MH (2012) Present status of the development of mycoherbicides against water hyacinth: Successes and challenges. A review. *Biotechnol, Agron, Soc Environ* 16(3):360-8
- Kieloch R, Domaradzki K (2011) The influence of some spraying parameters and herbicide formulation on its effectiveness. Pages 65-69 *in* Proceedings of the V International Scientific Symposium: Farm machinery and process management in sustainable agriculture. Lublin, Poland: Department of Machinery Exploitation and Management in Agricultural Engineering
- Kittock DL, Arle HF (1977) Termination of late season cotton fruiting with growth regulators. *Crop Sci* 17:320-324
- Kolota E, Chohura P, Komosa A (2013) Efficiency of chelate forms of micronutrients in nutrition of greenhouse tomato grown in rockwool. *J Elem* 18(4): 635-647
- Law Q, Patton, AJ, Weisenberger D (2012) Efficacy of current organic postemergent weed control options in turfgrass systems. Page 71552 *in* Proceedings of the ASA, CSSA and SSSA Annual Meetings. Cincinnati, Ohio
- Liu DLY, Christians NE, Garbutt JT (1994) Herbicidal activity of hydrolyzed corn gluten meal on three grass species under controlled environments. *J Plant Growth Regul* 13(4):221-6

- Martins JG, Neto IFF, Pinto ISS, Soares EV, Barros MT, Soares HMVM (2014) Alternative chelating agents: evaluation of the ready biodegradability and complexation properties. *J Environ Sci Health, Part A: Environ Sci Eng* 49: 344-354
- McDade MC, Christians NE (2000) Corn gluten meal--a natural preemergence herbicide: Effect on vegetable seedling survival and weed cover. *Am J Alternative Agric* 15(4):189
- Price WJ, Shafii B, Seefeldt SS (2012) Estimations of dose-response models for discrete and continuous data in weed science. *Weed Technol* 26:587-601
- Riddle G, Burpee L, Boland G (1991) Virulence of *Sclerotinia sclerotiorum* and *S. minor* on dandelion (*Taraxacum officinale*). *Weed Sci* 39:109-118
- Schnick PJ, Stewart-Wade S, Boland GJ (2002) 2,4-D and sclerotinia minor to control common dandelion. *Weed Sci.* 50(2):173-8
- Siva C (2014) Alternative strategies for broadleaf weed management in residential lawns. MS thesis. Guelph, ON. The University of Guelph.
https://atrium.lib.uoguelph.ca/xmlui/bitstream/handle/10214/8046/Siva_Cynthia_201405_Msc.pdf?sequence=1. Accessed: May 24, 2015. 157p
- St. John R, DeMuro N (2013) Efficacy of corn gluten meal for common dandelion and smooth crabgrass control compared to nitrogen fertilizers. *Appl Turfgrass Sci* DOI:10.1094/ATS-2013-0426-01-RS
- Thakore Y (2006) The biopesticide market for global agricultural use. *Ind Biotechnol* 2(3):194-208
- Tuti MD, Das TK (2010) Optimizing dose, volume rate and time of application upon post-emergence use of metribuzin for weed control in soybean. *Pestic Res J* 22(2):146-150
- Webber CL, Shrefler JW (2007) Pelargonic acid weed control: Concentrations, adjuvants, and application timing. Pages 145-148 *in* Proceedings of the 26th Oklahoma-Arkansas Horticultural Industry Show. Ft. Smith, Arkansas
- Webber CL, Taylor MJ, Shrefler JW (2014) Weed control in yellow squash using sequential postdirected applications of pelargonic acid. *HortTechnology* 24(1): 25-29
- Webster, TM (2012) Weed Survey – Grass Crops Subsection. Pages 267-288 *in* Proceedings of the Southern Weed Science Society. Charleston, SC: Southern Weed Science Society
- Wilen C (2012) Preliminary Report on Iron HEDTA: A Natural Selective Herbicide. Retail Nursery IPM Newsletter. <http://www.ipm.ucdavis.edu/PDF/PUBS/retailipmnews.2012.mar.pdf>. Accessed: July 10, 2014

Part IV

Effects of biopesticide products, Thaxtomin A, *Phoma macrostoma* and FeHEDTA on ornamental plants

Abstract:

Several experiments were conducted to evaluate the safety of Thaxtomin A, *Phoma macrostoma* and FeHEDTA on woody ornamental plants grown in containers. No injury was observed on from *Phoma macrostoma* treatments. Thaxtomin A and FeHEDTA injured the foliage of broadleaf ornamental plants when new growth was contacted by spray applications, but no injury was observed on juniper plants. On broadleaf evergreen plants, no injury to fully matured foliage or to dormant plants was observed, and new growth emerging after treatments was unaffected. These data suggest that “application windows” exist for use of FeHEDTA or thaxtomin A for broadleaf weed control in woody ornamental plants either as an over the top application when plants are not actively growing, or as a directed spray.

Materials and Methods

Experiments investigating the safety of bio-based herbicides on ornamental plants were conducted at the North Carolina State University Horticultural Field Laboratory in Raleigh, NC from 2011 to 2014. Rooted cuttings *Juniperus conferta* ‘Blue Pacific’, *Ligustrum japonica*, *Spiraea japonica* and *Gardenia radicans* were obtained from a local nursery. In each experiment, plants were arranged in a randomized complete block design with 4 replications in 2011 and 2012 and 6 replications in 2014. In the 2014 experiment, pots containing seedling *Oxalis stricta* (yellow woodsorrel) and *Cardamine flexuosa* (flexuous bittercress) were included to obtain concomitant efficacy data on two of the most common weeds of nursery crops..

Treatments in the 2011 and 2012 treatments included *P. macrostoma* at 6500 and 13000 mu/m², thaxtomin A at 380 g ai/ha, and FeHEDTA at 59 kg ai/ha. In 2012, an additional treatment of FeHEDTA at 30 kg ai/ha was also included. Initial treatments were applied on October 17 in 2011 and September 13 in 2012, and were made over the top of actively growing plants. In 2011 and 2012, treatments were re-applied four weeks after the initial applications on November 14, 2011 and October 16, 2012. In the 2014 experiment, treatments included thaxtomin A at 380 g ai/ha, FeHEDTA at 30 and 59 kg ai/ha, and an initial treatment of thaxtomin A at 380 g ai/ha followed by an application of FeHEDTA at 30 kg ai/ha two weeks later. In the 2014 experiment, treatments were applied to the plants while dormant on February 27, 2014. FeHEDTA was reapplied two weeks after initial treatments on March 13, 2014, and thaxtomin A was reapplied three weeks after initial treatments on March 20, 2014. At the time that Fe-HEDTA was re-applied, the gardenias were beginning to break dormancy; by the time MBI-005 was re-applied the following week, new growth was visible on the spirea, ligustrum and gardenias. *Phoma macrostoma* treatments were made using hand-held shaker jars. All other treatments were applied using a CO₂ pressurized backpack sprayer calibrated to deliver 50 GPA

In all experiments, pots were overhead-irrigated as needed. When needed, overhead irrigation was applied in 3 equal increments, early morning, noon and late afternoon for a total of about 0.5 inches of water per day. Plants were placed in an un-heated, plastic-covered shelter for winter

protection. In 2011 and 2014, phytotoxicity was visually evaluated beginning one week after initial applications and was evaluated weekly thereafter. Evaluations were conducted on a 0 to 10 scale where 0 = no damage (equal to the non-treated) and 10 = dead. Intermediate values are visual estimates of percent reduction in above ground plant biomass abbreviated to a 0 to 10 scale. In 2012, evaluations included visual estimations of overall plant tissue necrosis, which is presented in our results as percent necrosis.

Results

Phoma macrostoma did not cause injury to any of the four woody plants tested in 2011 or 2012. In 2011, spirea was the only species visibly injured by any treatment, and was injured 13 and 33% by thaxtomin A and FeHEDTA, respectively. Results in 2012 differed from those observed in 2011. Of the four woody ornamentals tested, spirea was the most sensitive to injury from both thaxtomin A and FeHEDTA with about 12% of the foliage necrotic or damaged following the 2nd application (See Table 1). Only new growth present at the time of application was affected. Older foliage was not affected, nor was new growth arising after treatment affected. Similarly, new growth on gardenia was damaged by thaxtomin A and FeHEDTA. Thaxtomin A caused discoloration of about 4% of the foliage; FeHEDTA caused necrosis of about 6% of the foliage. *Ligustrum japonicum* new growth was injured by FeHEDTA but was not injured by thaxtomin A. Juniper plants were not injured by any treatment.

Table 1. Phytotoxicity of bio-based weed control products from over the top applications to container grown woody ornamentals.

Treatment	Dose	Spirea	Ligustrum	Gardenia	Juniper
		----- % foliar necrosis -----			
Non-Treated		1.5 c	0 b	0 c	0
<i>P. macrostoma</i>	6500 <i>mu</i> /m ²	0 c	0 b	0 c	0
<i>P. macrostoma</i>	13000 <i>mu</i> /m ²	0 c	0 b	0 c	0
Thaxtomin A	380 g ai/ha	11.3 abc	0 b	3.8 ab	0
FeHEDTA	30 kg ai/ha	4.0 bc	7.4 a	2.5 bc	0
FeHEDTA	59 kg ai/ha	11.5 a	8.0 a	6.3 a	0

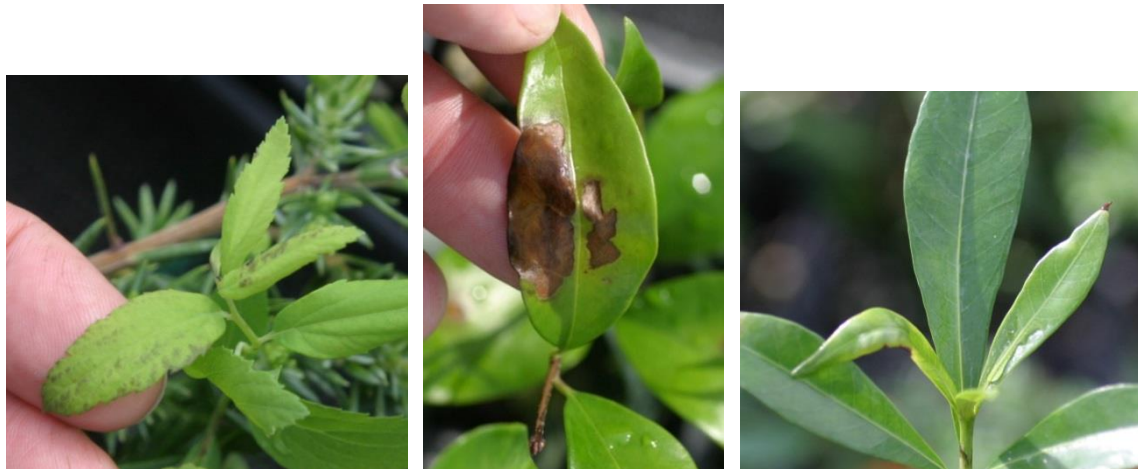
In 2014, within 2 weeks of the initial treatment, foliar injury was visible on the ligustrum and gardenias treated with both rates of FeHEDTA. Symptoms were present as small brown spots on the ligustrum and slightly larger necrotic spots on gardenias. Foliar injury never exceeded 18% on either species at either rate of FeHEDTA (See Table 2).

Table 2. Phytotoxicity of bio-based weed control products from over the top applications to container grown woody ornamentals. Evaluation conducted 1 week after final treatment.

Treatment	Dose	Spirea		Ligustrum		Gardenia		Juniper
		4 WAT	6 WAT	4 WAT	6 WAT	4 WAT	6 WAT	
Thaxtomin A	380 g ai/ha	13 a	0	0 c	0 c	10 a	8 b	0
FeHEDTA	30 kg ai/ha	10 a	0	9 ab	10 ab	10 a	18 ab	0
FeHEDTA	59 kg ai/ha	20 a	0	15 a	15 a	16 a	23 a	0
Thaxtomin A fb	380 g ai/ha	18 a	0	3 bc	3 bc	11 a	13 ab	0
FeHEDTA	30 kg ai/ha							

WAT = weeks after the initial treatment. 4 WAT was 4 weeks after the initial treatment and 1 week after the final treatment. Note: no injury was observed on any evaluate date on junipers.

Shortly after the second application of thaxtomin A, injury symptoms were observed on treated gardenias. Unlike the injury symptoms observed on plants treated with FeHEDTA, injury from thaxtomin A presented as irregular, epinastic new growth emerging from growing points. These symptoms were not present on any plants other than the gardenias, and were present on both plants that received 2 applications thaxtomin A at 380 g ai/ha, and plants which received 1 application of thaxtomin A at 380 g ai/ha followed by FeHEDTA at 30 kg ai/ha. Because the gardenias were not visibly breaking dormancy until after the initial application, this may imply that injury due to thaxtomin A applications are due to activity in the root zone rather than a foliar mode of action.



Photos: left to right –phytotoxicity from over the top applications of FeHEDTA to spirea, ligustrum and gardenia new growth.

New growth observed after this evaluation did not appear to be affected by any of the treatments, and plants which exhibited injury following applications of thaxtomin A eventually recovered and resumed new growth. FeHEDTA treatments resulted in necrosis of the new growth on gardenia, spirea and ligustrum, but new growth was unaffected. The following spring when plants resumed growth, no treatment caused visible injury. Foliage on evergreen plants

(gardenia and ligustrum) damaged by FeHEDTA was persistent and still visible the following spring, but new growth was unaffected.

Seedling yellow woodsorrel was controlled by both thaxtomin A and Fe-HEDTA (Table 3). Increasing the FeHEDTA dose from 30 to 59 kg ai/ha improved control to over 95%. Seedling flexuous bittercress was well controlled by thaxtomin A but Fe-HEDTA was less effective. These results are consistent with data reported in Part I of this document.

Table 3. Efficacy of bio-based weed control products on common nursery weeds, evaluated 4 weeks after initial treatment.

Treatment	Dose	Bittercress	Woodsorrel
		----- % control -----	
Thaxtomin A	380 g ai/ha	85 a	83 b
FeHEDTA	30 kg ai/ha	30 c	80 b
FeHEDTA	59 kg ai/ha	33 c	96 a
Thaxtomin A fb FeHEDTA	380 g ai/ha fb 30 kg ai/ha	55 b	86 ab

Based on these data, we can conclude that foliar injury is likely when thaxtomin A or FeHEDTA are applied over the top of actively growing broadleaf ornamentals. However, when applied over the top of dormant ornamentals neither thaxtomin A nor FeHEDTA caused significant injury. These data also suggest that treatments would likely not be injurious when applied as a directed spray (avoiding contact with the foliage) or over the top of plants that do not have a flush of tender foliage present at the time of treatment. No injury was observed on junipers from any of the three biopesticides tested. Thus we can conclude that FeHEDTA or thaxtomin A have potential for selective postemergence control of certain broadleaf weeds in production. Additionally these data support the conclusion that properly applied turfgrass applications would have little potential for off-target damage to nearby ornamental plantings even when broadleaf ornamental plants are actively growing.

Part V

Bio-based product comparison for the control of dicondra in turf

Objective

Previous research demonstrated that *Phoma macrostoma* controlled *Trifolium repens* (white clover), a creeping perennial broadleaf weed and suppressed other dicot weeds. Similarly, FeHEDTA provided excellent control of white clover but plants recovered when stolons were not completely killed. *Dicondra carolinensis* (dicondra) is another common stoloniferous weed of southern turf. In our prior experiments, small patches of dicondra were present in some plots. In those situations we observed significant injury to dicondra from several bio-based treatments but populations were insufficient to draw conclusions about product efficacy. Thus, an experiment was conducted to compare *P. macrostoma*, MBI-005, Fe-HEDTA and Weed B Gon Max for the control of in turf.

On November 21st, 2012 an established stand *Dichondra carolinensis* (DIORC) was treated with 110 g/m² *Phoma macrostoma* formulation EP02-13-2012 (containing about 120 µg of macrocidin); 38 L/ha MBI-005, 160 L/ha Fiesta (Fe-HEDTA) and the manufacturer's recommended dose of Weed B Gon Max ready to use (RTU) herbicide -- Weed B Gone Max (~42 ml / m²). The experiment was repeated in 2013 with the following adjustments. MBI-005 was ineffective in 2012 and was not included. *Phoma macrostoma* was highly effective at the single dose tested, thus in 2013 *Phoma macrostoma*, formulation EP111312 was applied at 32.5, 16.25, and 8.125 g/m² (approximately equivalent to, half, and one-quarter of the dose of active mycotoxin applied in 2012. In 2012 regrowth from Fe-HEDTA treatment was evident shortly after treatment; thus, in 2013 a 2nd application was made 3 weeks after the first.

Treated plot size was 0.5 x 1 m. At the time of treatment the area the ground cover in each plot was over 90% dicondra with a small amount of tall fescue, common bermudagrass and white clover. Dicondra was not actively growing at the time of treatment. In 2012, Fe-HEDTA and MBI-005 were applied at 100 GPA spray volume by making two passes with a CO₂ pressurized sprayer equipped with a single 8008 flat fan spray tip and calibrated to deliver 50 GPA. Other experiments suggested that a higher spray volume may improve efficacy of Fe-HEDTA; therefore in 2013, the spray volume was increased to 200 GPA for Fe-HEDTA treatments (4 passes with a sprayer calibrated to deliver 50 GPA) (note: other research subsequently demonstrated that spray volume did not influence FeHEDTA efficacy).. Weed control was evaluated periodically following treatment with final evaluations made on March 15, 2013 and March 21, 2014. Weed control was visually evaluated on a 0 to 10 scale where 0 = no control and 10 = 100% control. Before treatment and at the termination of the experiment percent ground cover of living dicondra was estimated. About a month after the 2012 treatments, white clover in the *Phoma macrostoma* – treated plots were chlorotic but appeared to have elongated petioles; therefore, average height of the top of the plant canopy was measured.

MBI-005 did not control dicondra (Figure 1); therefore, this treatment was omitted from the 2013-14 experiment. Fe-HEDTA caused rapid and nearly complete necrosis of the dicondra

foliage in 2012, with over 90% foliar necrosis within one week after treatment. Regrowth occurred and by spring only about 30% control was observed (Figure 1). In 2013, the amount of necrosis caused by FeHEDTA was significantly less and there was no difference between single or multiple applications in the fall. In the spring, dicondra regrowth was observed in the Fe-HEDTA treated plots with about 80% and 40% ground cover of dicondra in March 2013 and 2014, respectively (Figure 3). In this experiment, no additional treatments were applied in the spring when growth resumed. Repeated applications would be necessary to provide more complete control. In contrast, *Phoma macrostoma* caused only slight chlorosis of dicondra in the fall but in the spring little re-growth was observed (Figures 1 and 2). In March of each year, dicondra control with *Phoma macrostoma* was equivalent to that provided by the commercial standard herbicide (Figures 1-4). In 2014, no significant differences between the three doses of *Phoma macrostoma* were observed for percent control evaluations. However, there was a dose response for percent cover evaluations with the full, half and quarter doses resulting in about 4, 6, and 14% ground cover of dicondra, respectively. In comparison, the non-treated plots had about 64% cover. Weed B Gon Max caused typical auxinic herbicide symptoms on dicondra in the fall but over 90% control and less than 4% ground dicondra ground cover by March each year.

These data demonstrate that dicondra is particularly sensitive to *Phoma macrostoma*, with low doses of this biological control agent providing control equivalent to the industry standard herbicide treatment. Fe-HEDTA is effective on dicondra, but appears to have primarily a contact-type action and would require repeated applications to obtain acceptable control.

Figure 1. Percent control of *Dicondra carolinensis*; treated November 21, 2012; evaluated December 19, 2012 and March 15, 2013.

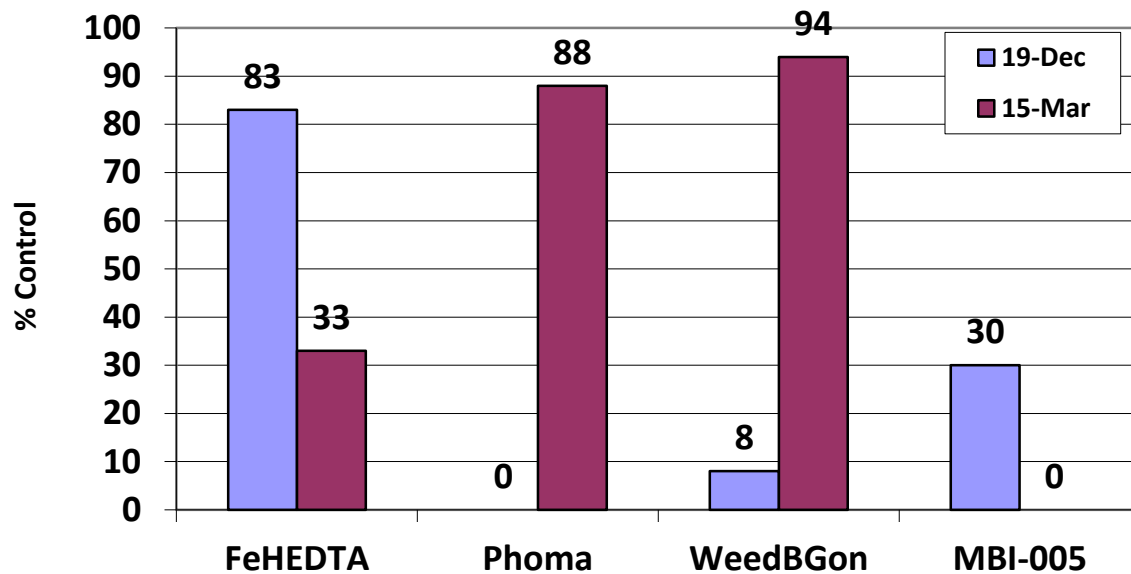


Figure 2. Percent control of *Dicondra carolinensis*; treated November 6, 2013; evaluated November 22, 2013 and March 21, 2014.

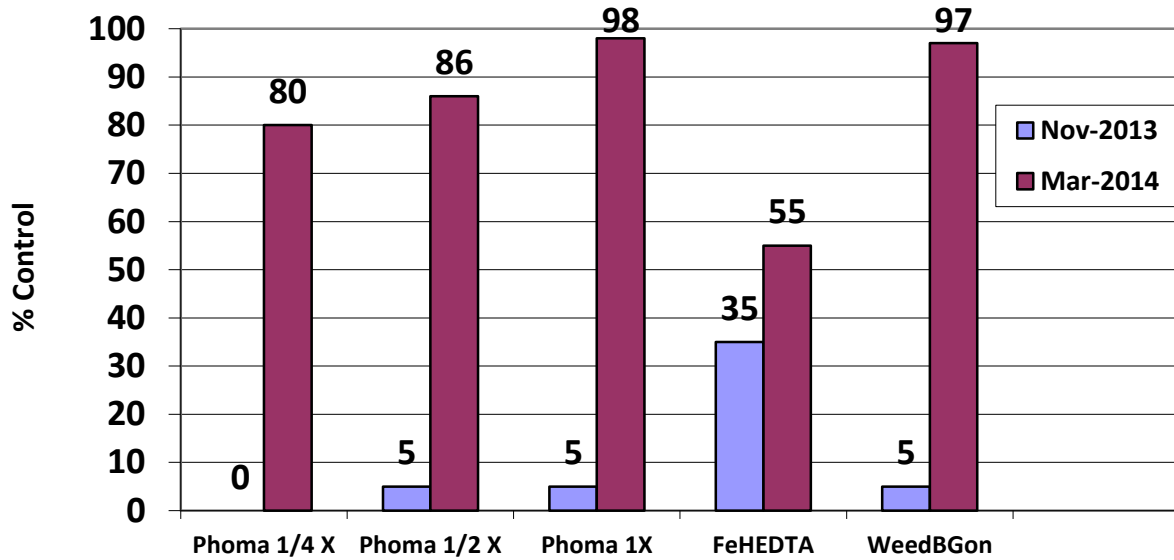


Figure 3. Percent ground cover of *Dicondra carolinensis*; treated November 2012 and 2013; evaluated March 2013 and 2014.

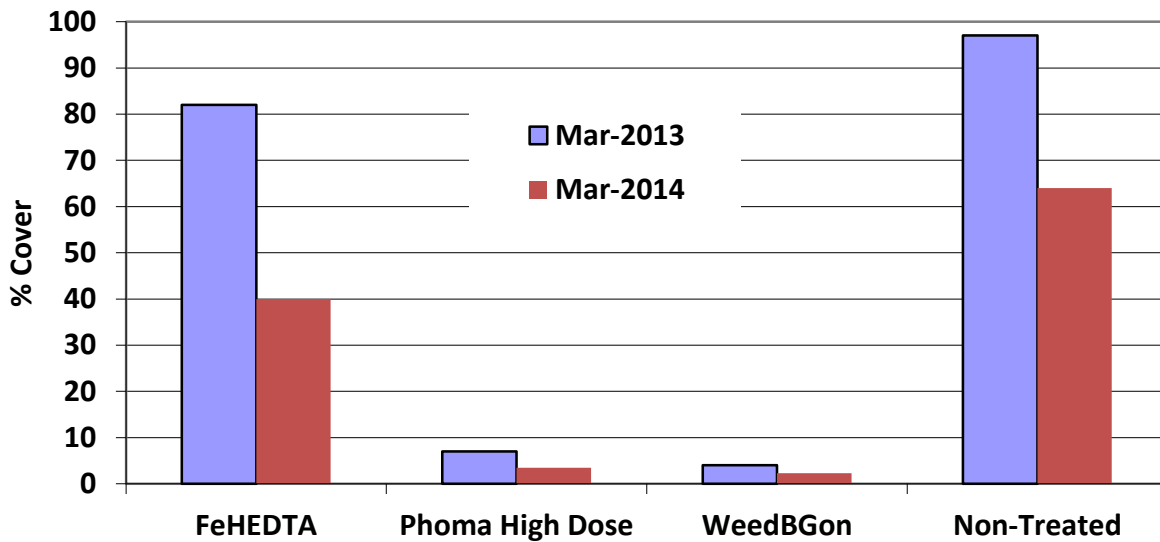


Photo 1. Dicentra control: Weed B Gon Max on left. *Phoma macrostoma* treated plot on the right. Photo taken mid-February following a November treatment.



Photo 2. Dicentra control with *Phoma macrostoma*. Treated November 21, 2012; Photo taken March 13, 2013. *Phoma* – treated plot on the left, non-treated on the right.

