ABSTRACT

WOLFE, JOSEPH CARLETON. Selective Weed Control in Turfgrass Using the Bioherbicides *Phoma macrostoma*, Thaxtomin A and FeHEDTA. (Under the direction of Dr. Joseph C. Neal).

The worldwide demand and market for biopesticides has increased significantly in recent years, particularly in amenity areas like turfgrass. In spite of the demand, few natural products are available for weed control in turfgrass. Field and container experiments were conducted to evaluate the weed control efficacy of three bioherbicides which have been reported to selectively control broadleaf weeds in turfgrass. *Phoma macrostoma*, a fungal pathogen isolated from Canada thistle, controlled container-grown dandelion (*Taraxacum officinale*), marsh yellowcress (*Rorippa palustris*), and flexuous bittercress (*Cardamine flexuosa*) equivalent to an industry standard herbicide, pendimethalin, when applied PRE, but did not when applied POST. PRE applications of thaxtomin A, a compound produced by the bacterium *Streptomyces scabies*, controlled dandelion, marsh yellowcress, flexuous bittercress, yellow woodsorrel (*Oxalis stricta*), ivyleaf speedwell (*Veronica hederifolia*), and annual bluegrass (*Poa annua*) equal to pendimethalin in at least one of two tests, but did not control henbit (*Lamium amplexicaule*) or common chickweed (*Stellaria media*) in containers. POST applications of thaxtomin A controlled six of eight species tested as well as an industry standard auxinic herbicide. In field tests, overall PRE broadleaf weed control with *Phoma macrostoma* and thaxtomin A was 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 in turf using *Phoma 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; PRE followed by early POST applications of thaxtomin A provided $\geq$ 86%, henbit control, equal to pendimethalin. Additional studies were conducted to evaluate the efficacy of PRE applications of thaxtomin A on annual bluegrass and smooth crabgrass (*Digitaria ischaemum*). When applied at 380 g ai ha^{-1} and four-week intervals, thaxtomin A controlled smooth crabgrass equal to pendimethalin through July, but control declined rapidly when treatments ceased. One application 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 pendimethalin. Thaxtomin A safety on tall fescue (*Lolium arundinaceum*) and perennial ryegrass (*Lolium perenne*) was also evaluated. When applied at 380 g ai ha^{-1}, thaxtomin A reduced tall fescue and perennial ryegrass cover when applied one week before seeding, at seeding, and one week after seeding, but was safe at other timings. Up to three applications of thaxtomin A at 380 g ai ha^{-1} at four week intervals did not reduce perennial ryegrass cover. FeHEDTA was evaluated for POST broadleaf weed control. 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. In at least one year of container experiments, FeHEDTA provided control equal to that of an industry standard synthetic auxin herbicide for five of the seven weed species tested, but did not control henbit or common chickweed. Results in newly-seeded turf were similar, with $<$ 30% control of common chickweed. In a separate experiment, three applications of 59.4 kg ai ha^{-1} FeHEDTA at two week intervals provided 100% control of henbit and 79-92% control of

common chickweed. These results suggest that these bioherbicides are capable of controlling certain broadleaf weeds in turfgrass, but all lack efficacy on some regionally important weed species. Control programs utilizing these bioherbicides may allow turf managers to provide commercially acceptable control of several weed species in areas where they are unable or prefer not to utilize synthetic herbicides.

Selective Weed Control in Turfgrass Using the Bioherbicides *Phoma macrostoma*, Thaxtomin A and FeHEDTA

by
Joseph Carleton Wolfe

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Horticultural Science

Raleigh, North Carolina

2015

APPROVED BY:

Dr. Joseph C. Neal Committee Chair	Dr. Fred H. Yelverton Committee Member

Dr. Travis W. Gannon
Committee Member

DEDICATION

This thesis is dedicated to my parents, Carleton Joseph Wolfe and Linda Lee Wolfe, for providing the love and support I needed to become the person I am today. Thank you for your patience, your understanding, and your dedication to providing the best upbringing and home environment I could have ever hoped for. For as much pride as you have always had in my achievements, I am equally proud to have been raised by such kind and loving parents.

BIOGRAPHY

Joseph Carleton Wolfe was born on November 23, 1986, in Philadelphia, Pennsylvania to Carleton and Linda Wolfe. He grew up in the nearby suburb of Fairless Hills, enjoying the outdoors, reading, writing, family, and friends. After high school Joseph attended The Pennsylvania State University, where he decided to pursue a career that would allow him to spend time outdoors during his working day. After earning a Bachelor of Science degree in Horticultural Science in 2010, he relocated to North Carolina and worked briefly in the nursery industry as a Section Grower. Inspired by his love of science and a desire to learn, Joseph accepted a position as a Research Associate with Bayer CropScience. In his new position, he learned the fundamentals of weed science and discovered a passion for scientific research. While employed at Bayer CropScience, Joseph was encouraged by his mentors and coworkers to pursue graduate school. In August, 2013, he began working toward his Master of Science degree as North Carolina State University under the direction of Dr. Joe Neal, with a focus on weed control in turfgrass and ornamentals. Upon completion, Joseph will continue his Ph.D. graduate work at North Carolina State University, and hopes to one day pursue a career in herbicide development for turfgrass and ornamental crops.

ACKNOWLEDGMENTS

I would like to express my sincere appreciation to my graduate committee: Dr. Joe Neal, Dr. Travis Gannon, and Dr. Fred Yelverton for their assistance and support throughout my graduate studies. I would especially like to thank Dr. Joe Neal for his guidance, encouragement, and patience throughout my educational experience. I would also like to specifically thank Dr. Travis Gannon for his assistance in finding and securing suitable areas for experiments. I would like to extend a special thanks to Dr. Chris Harlow, whose technical support, advice, and company was invaluable to me throughout my research. The statistical expertise of Dr. Consuelo Arellano was also greatly appreciated. Additionally, I would like to express my gratitude to all of the technical support staff who assisted in the establishment and maintenance of my experiments, including, but not limited to, Bradley Holland, Tim Ketchie, Matthew Jeffries, Benjamin Pease, Dustin Corbett, and John Michael Phillips. Finally, a very special thanks to my family, for their encouragement and support over the last two years.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SCOPE AND JUSTIFICATION

One of the earliest definitions of a weed, and one which is still commonly used today, is "a plant out of place or growing where it is not desired" (Zimdahl 2013a). Traditionally, the impact of weeds in agriculture has been associated with reduced yield and crop quality due to competition for limited resources, primarily light and water. In non-agricultural settings, weed management is also frequently employed to reduce human health risks associated with allergies and to improve aesthetics; for instance, in turfgrass systems the presence of unsightly weeds can reduce visual and textural uniformity, while in landscapes they may distract from ornamental plants and other landscape features.

Weed management has been a concern for farmers for centuries, though prior to the year 1900 weed removal was primarily accomplished either by hand or through tillage for seedbed preparation. The earliest documented uses of herbicides occurred in Germany, and included the use of lime to control horsetail (*Equisetum sp.*) in 1840, and the use of salt to control a variety of weed species in 1854 (Timmons 2005). Research into potential inorganic chemicals to control weed populations began in the 1890s in Europe, and interest in the field grew rapidly until the early 1940s (Timmons 2005). The discovery of the herbicidal activity of the phenoxyacetic acids and the development and commercialization of 2,4-D between 1942 and 1944 is sometimes considered the beginning of the "Chemical Era of Agriculture" (Timmons 2005).

Industry Overview

As the world population grew during the 20th century the use of synthetic chemical fertilizers and herbicides reduced production costs and increased yields, thus making them ubiquitous in agriculture. The so-called “green revolution,” which greatly increased the ability of farmers to produce food to sustain an increasing world population, came about largely due to a combination of modern plant breeding strategies and the usage of synthetic fertilizers and pesticides (Zimdahl 2013b). In addition to their widespread use in agriculture, synthetic herbicides also became popular among landscapers, homeowners, and governmental agencies due to their ability to improve aesthetics and cost-effectively manage unwanted vegetation. Global pesticide expenditures in 2007 were estimated at $39.7 billion, with 39% being spent on herbicides (Grube et al. 2011). While this amount is substantial, the market for synthetic pesticides has seen a gradual decline since 2001, largely due to greater demand for more environmentally friendly pesticides, the introduction of genetically modified crops, newer, lower-dose products, and the increasing trend toward organic farming (Thakore 2006). Biopesticides currently make up only a small portion of the worldwide pesticide market, with a total estimated value of $460 million in 2000 (Bailey and Mupondwa 2006). Sales have continued to increase yearly, however, with projected sales expected to reach over $1 billion by 2010 (Bailey et al 2010).

One market segment which has seen both consumer opinion and regulatory forces pushing strongly for alternative pesticides is in the turfgrass industry. In the US alone, turfgrass occupies more than 30 million acres of land, including some 50 million lawns in addition to golf courses, athletic fields, parks, and sod farms; between services and associated products, turfgrass in the US is an industry with total annual sales estimated at $45 billion (Grewal 1999). In 1996 over $200 million was spent on herbicides for use in highly maintained turf in the United States, with expenditures expected to increase by 2-4% annually (Porpiglia et al. 1996).

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 Europe, widespread safety concerns have led to a large number of pesticides being banned or heavily restricted, particularly applications being made for aesthetics. Similarly, the governments of Quebec and Ontario have enacted bans on the usage of any pesticides in amenity areas in 2003 and 2009, respectively (Bélair et al. 2010). Similar regulatory restrictions are not as widespread in the United States as those in Canada, but do exist. In 2011, the state of New York banned all synthetic pesticide usage on school properties (Grant 2011). Some individual municipalities have gone even farther, such as Suffolk County on Long Island, which has mandated that all county property must be managed organically (Bélair et al. 2010).

In addition to the influence of regulatory forces, the increasing prevalence of herbicide resistance in weed populations is a growing concern which demands investigation into new management strategies. As of 2012, there were 372 known biotypes of herbicide-resistant weed species confirmed worldwide (Vencill et al. 2012). The number of herbicide-resistant biotypes has increased dramatically since the introduction of genetically modified, herbicide-resistant crops, which led to heavy reliance on a single mechanism of action for weed control in many cropping systems (Norsworthy et al. 2012). Concerns about environmental, health and safety, and management of resistant weed populations; as well as regulatory restrictions on synthetic pesticide use in urban areas have led to increased interest in developing alternative weed management strategies. Specifically, biological control strategies and reduced risk biopesticides for weed control in turfgrass systems have received considerable attention (Bailey et al. 2011; Belair et al. 2010; Boyetchko et al. 2009)

Biological Control

Biological control has gained considerable attention in recent years as a potential alternative to synthetic pesticides. “Classical” biological control is typically focused on introducing natural enemies to reduce pest populations to low levels while also maintaining a stable population of the natural enemy (Boyetchko et al. 2009). In the case of weed control, this generally entails identifying highly selective arthropod predators from the plant’s native region and releasing them into the environment to establish and spread. Theoretically, this strategy allows for long-term weed suppression and requires minimal financial investment. Successful examples of this biological control strategy include common St. Johnswort (*Hypericum perforatum*) control using *Chrystolina* leaf beetles, and diffuse and spotted knapweed (*Centaurea diffusa* and *Centaurea stoebe*) control with a combination of 12 insect predators. However, it is estimated that only about one third of the biological weed control agents screened and released in Canada have had any impact on weed populations (Boyetchko et al. 2009).

An alternative to classical biological control is the inundative or biopesticide approach, which involves application of biocontrol agents at high doses in order to rapidly and completely control the pest but does not rely upon the biopesticide persisting in the environment. Biopesticides, by their broadest and most widely accepted definition, are a class of biocontrol agents, including living organisms and natural products derived from those organisms, which suppress pest populations (Bailey et al. 2010). The United States Environmental Protection Agency defines a biopesticide as "pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals (Anonymous 2015a).

Since interest in bioherbicides became widespread in the 1980s, research has primarily focused on the use of active microbial agents, primarily plant pathogens, to control invasive weeds (Hoagland 1990). In 1992 BioMal (*Colletotrichum gloeosporioides f.*sp.*Malvae*), a fungal pathogen, was the first bioherbicide registered in North America, and was intended to control common mallow (*Malva neglecta*) in field crops (Boyetchko et al. 2009). Since that time, a common research strategy has been to isolate host-specific pathogens from the target weed.

This approach has produced several other agents with high specificity and high levels of efficacy on target species, such as DeVine (*Phytophthora palmivora*), which provided 95-

100% control of stranglervine (*Morrenia odorata*) for up to 1 year after application (Kenney, 1986). Likewise, Collego (*Colletotrichum gloeosporioides f.* sp. *Aeschynomene*), which was developed between 1982 and 2003, was shown to offer up to 80% control of northern jointvetch (*Aeschynomene virginica*) in rice and soybeans (Dittmore et al. 2008).

This approach does have limitations. Such high levels of host specificity can lead to difficulties in commercialization. DeVine is no longer in production due to a relatively small market for the product and the high cost of maintaining registration (Karim Dagno et al. 2012). Production of Collego was halted after 2003 for similar reasons, though small amounts of the product were brought back onto the market in 2014 (Boyette et al. 2014; Karim Dagno et al. 2012). Likewise, while BioMal remains registered in Canada and can still be obtained in small quantities, large-scale commercial production was halted in 1994 when it was determined that newer, less expensive chemicals capable of controlling common mallow had been introduced over the 10 year development period and had significantly reduced the product's potential profitability (Boyetchko et al. 2009; Karim Dagno et al. 2012).

Although high degrees of host specificity is generally considered desirable, this limits a biocontrol agent's potential market. An alternative strategy which has received significant attention is the potential utilization of broad-host range pathogens for broadleaf weed control in grass crops (Riddle 1991, Bourdot 2011).

Other common issues which have been observed when attempting to utilize live pathogens as bioherbicides involve biological limitations; for example, their efficacy is frequently influenced by environmental conditions at the time of application, and live organisms often have limited shelf lives and are not ideally suited for long-term storage (Charudattan and Dinoor 2000; Ghosheh 2005). A good example of the potential limitations caused by environmental factors was *Xanthomonas campestris* pv. *poannua*, a pathogen which causes bacterial wilt of annual bluegrass. Despite showing some promise in controlled environments, *Xanthomonas campestris* pv. *poannua* was not successfully commercialized due to low performance and variability in efficacy when applied under different environmental conditions (Johnson 1994; Zhou and Neal 1995).

Because of these and other limitations, relatively few bioherbicides have been successfully registered and commercialized in North America despite increasing demands for more environmentally-friendly weed control. In a 2005 retrospective review of bioherbicide development projects, it was estimated that only 8.1% of bioherbicides which enter development have been registered and used with any regularity in their region (Charudattan 2005). While several bioherbicides with high degrees of efficacy have been identified and developed in recent years, there are several major factors which must be addressed in their development. Past failures have often occurred due to difficulties in the transition from product development into the marketplace, primarily due to the high costs of registration and large-scale production relative to their potential market (Jarvis et al. 2007; Pacanoski 2011).

The high costs associated with bioherbicide development have frequently deterred investors from supporting bioherbicide projects, and in other cases has caused conflicts between researchers and investors (Ash 2010; Charudattan 2005). These potential problems emphasize the need for cooperation between researchers and industry in order to identify bioherbicides which offer both sufficient market potential and safe, effective weed control.

Despite these challenges, as of 2015, five bioherbicides are both registered and available in North America. Of these, three can be characterized as highly host-specific: the aforementioned BioMal and Collego, which remain in limited production, and Smolder (*Alternaria destruens*), which is labelled for the control of dodder species (*Cuscuta spp.*). The two other bioherbicides, Sarritor (*Sclerotinia minor*) and Chontrol (*Chondrostereum purpureum*) target a broad host range (Boyette et al. 2014; Karim Dagno et al. 2012). Other potential bioherbicides, such as the fungal pathogen *Phoma macrostoma* and SolviNix, an isolate of *Tobacco mild green mosaic tobamovirus* (TMGMV), have been registered by the US EPA but are not yet commercially available (Anonymous 2015b; Pitt et al. 2012)

Sarritor, a product which was commercialized in Canada as a bioherbicide for control of dandelion in turf, is a good example of the current trends in bioherbicide development. *Sclerotinia minor* is a highly virulent, polyphagous plant pathogen, which offered significant chances of off-target injury when used as an herbicide (Riddle et al. 1991). Its broad host range, however, made *Sclerotinia minor* an attractive bioherbicide candidate, creating significantly greater market potential than past bioherbicides. Chontrol, another recently-developed bioherbicide, is a saprophytic fungus with a similarly broad host range; it can be used to control a large number of hardwood tree species, and is used for vegetation management in rights-of-ways (Boyetchko et al. 2009).

In spite of their greater marketability due to a broader host range, both Sarritor and Chontrol faced difficulties in the development process due to the potential for off-target injury. In both cases, regulatory concerns have been raised over the potential long-term effects on ecosystems that could result following the widespread release of polyphagous pathogens. For these reasons, the research on both Sarritor and Chontrol demonstrate the importance of thorough investigation into the environmental fate of bioherbicides which exhibit a broad spectrum of control (Bailey and Mupondwa 2006; Bourdôt et al. 2011).

The Future of Bioherbicides in Turfgrass

A significant portion of recent bioherbicide research in turfgrass has focused on the identification and characterization of bioherbicides with broad host ranges, several of which also exhibit the ability to selectively control broadleaf weeds while remaining safe on grasses (Boyetchko et al. 2009; Siva 2014). Some of these have included pathogens such as Sarritor (*Sclerotinia minor*) and *Phoma macrostoma*, others utilize toxins produced by pathogens such as thaxtomin A, and still others include metal ion chelates such as FeHEDTA. While Sarritor has not yet been introduced in the United States for testing, the other three options have all shown promise in a small number of experiments.

***Phoma macrostoma*.** *Phoma macrostoma* is a coelomycete fungal pathogen isolated from Canada thistle (*Cirsium arvense*), and has been shown to cause bleaching, chlorosis, and eventual death in a variety of broadleaf weed species, yet is nonpathogenic to monocots (Bailey et al. 2009; Evans et al. 2013). Bailey et al (2011) reported 38 economically important dicotyledonous weed species from 12 families to be susceptible to infection by *Phoma macrostoma*. *Phoma macrostoma* reduced growth of both roots and shoots along with severe bleaching (Graupner et al. 2006). Though its mode of action is still not fully understood, the symptoms induced on susceptible species are similar to that of non-photosynthetic pigment-inhibiting herbicides (Bailey et al. 2009; Evans et al. 2013).

Because of the pathogen's ability to control broadleaf weeds without harming grass species, *Phoma macrostoma* was successfully registered for broadleaf weed control in turfgrass in 2011 and 2012 in Canada and the United States, respectively, though it is not yet commercially available (Pitt et al. 2012).

In keeping with the high level of regulatory concern over the potential environmental effects of releasing pathogens into new and potentially vulnerable ecosystems, research into the environmental fate of *Phoma macrostoma* has also been conducted. Thus far, research conducted on *Phoma macrostoma* has demonstrated minimal risk for injury to non-target species and limited soil mobility and persistence (Bailey et al. 2011; LeCong et al. 2004). More recent testing has focused on determining the influence of environmental factors such as temperature and fertility on the efficacy of *Phoma macrostoma*. Bailey et. al (2013) reported that control of dandelion (*Taraxacum officinale*) may be improved by 10 to 20% when *Phoma macrostoma* is applied in tandem with nitrogen fertilizers, while Neal et. al (2013) demonstrated that high temperatures may increase the efficacy of *Phoma macrostoma* on certain weed species, such as common groundsel (*Senecio vulgaris*). These findings suggest that the efficacy of *Phoma macrostoma* may be influenced by environmental factors at the time of application.

Though *Phoma macrostoma* possesses many characteristics desirable for a bioherbicide, the full extent of its applications in turfgrass have not been explored. Thus far research on *Phoma macrostoma* has been conducted almost exclusively in Canada, and thus no data is available on the ability of *Phoma macrostoma* to control many turfgrass weeds commonly found in the southern United States. Additionally, as Neal et al. (2013) and Bailey et al. (2013) suggested that environmental conditions can affect the ability of *Phoma macrostoma* to effectively control target weeds, further testing of *Phoma macrostoma* in turfgrass systems in the southern United States is necessary in order to account for environmental and climatic variations between regions.

Thaxtomin A. Thaxtomin A is one of a class of compounds known as thaxtomins, toxins produced by the bacterium *Streptomyces scabies*, the causal organism of common scab disease in potatoes (King et al. 1992; King et al. 2001). Screening processes used to search for potential herbicidally active compounds identified thaxtomin A as having activity similar to that of cellulose biosynthesis inhibitor herbicides, causing seedling stunting, cellular hypertrophy, and cell wall lignification (Duke and Dayan 2011; Fry and Loria 2002). A limited number of trials were conducted by King et al. (2001), showing that thaxtomin A is non-systemic but potentially highly phytotoxic. Their data indicated that thaxtomin A has more activity on dicotyledonous species than on monocots and is more active when applied PRE than when applied POST. Over the course of their trials, King et al. (2001) documented seedling stunting, meristematic injury and other symptoms consistent with cellulose biosynthesis inhibiting herbicides, but also observed wilting of all tested species, leading them to postulate that thaxtomin A may have a secondary herbicidal mode of action which may present with a different set of symptoms. The greater activity of thaxtomin A on dicotyledonous species, combined with its purported mode of action, suggests that applications in turfgrass may be feasible, as synthetic cellulose biosynthesis inhibiting herbicides have been available for many years and are utilized in turfgrass (Anonymous 2010; Sabba and Vaughn 1999).

Existing data characterizing the herbicidal activity of thaxtomin A, along with information regarding its environmental fate, are scarce. The initial experiments conducted on thaxtomin A's herbicidal activity were limited to a small number of model species, thus further research into its ability to control weed species of economic importance is required in order to evaluate its potential use as a bioherbicide. Furthermore, because thaxtomin A has received very limited testing outside of lab and greenhouse settings, additional research into thaxtomin

A's performance and persistence under field conditions is necessary prior to commercialization.

FeHEDTA. Historically, both ferrous sulfate and iron chelates have been used for control of moss and algae 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 is necessary in order for it to be successfully absorbed 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 is labelled for broadleaf weed control in turf (Anonymous 2009). While its mechanism of action and selectivity is not yet fully understood, it is possible that iron absorbed as FeHEDTA can function as a catalyst for oxygen reduction, leading to the formation of highly reactive oxygen species capable of causing cellular damage (Anonymous 2010). Yet, little information is available on FeHEDTA's efficacy and safety in turfgrass systems. 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 in three week intervals at 1000, 2000 and 4000 L ha^{-1} spray volumes, respectively, 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. Another series of tests was conducted by Siva (2014), and showed that two applications of FeHEDTA at 1 g ai m^{-2} applied in a 4000 L ha^{-1} spray volume can reduce cover of broadleaf weeds such as common dandelion, white clover, and black medic (*Medicago lupuplina*) to less than 5%. Similarly, Wilen (2012) reported that POST applications of FeHEDTA selectively controlled a large number of broadleaf weeds, causing foliar necrosis in target species within three days of application without injuring turf.

An experiment conducted by Law et al. (2012) did not produce acceptable weed control with FeHEDTA and 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. When compared to the control reported by Carey et al. (2010a, 2011a) and Siva (2014), these findings suggest that multiple applications of FeHEDTA may be necessary to significantly reduce weed populations and that certain weed species may be less susceptible to FeHEDTA injury than others. A recent literature search revealed no refereed publications detailing the efficacy of FeHEDTA on weed species other than those mentioned above. Of the 14 broadleaf species included in a 2012 survey of the most common weeds in turf in the southern United States, 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 fine 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}, re-applied three to four weeks later (Anonymous 2013). These guidelines offer a large range of dosing and cost per use which require further exploration. Past research into organic POST contact herbicides such as pelargonic acid, acetic acid, and pine oil demonstrated that factors such as carrier volume, herbicide concentration, and application timing can have a highly significant impact on their performance (Johnson et al. 2004; Webber and Shefler 2007). 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.

Objectives

The overall objective of this project was to evaluate *Phoma macrostoma*, thaxtomin A, and FeHEDTA as bioherbicides for use in turfgrass systems. Specific objectives of these studies were to: (1) characterize the efficacy of PRE and POST applications of *Phoma macrostoma* and thaxtomin A on broadleaf weeds, (2) evaluate thaxtomin A for PRE control of annual grassy weeds, (3) investigate the weed control spectrum and effects of dosing and application parameters on the efficacy of FeHEDTA.

Literature Cited

Anonymous (2008) Which pesticides are banned in Europe? http://www.pan-europe.info/Resources/Links/Banned_in_the_EU.pdf. Accessed June 3, 2015

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

Anonymous (2015a) US OKs BioProdex's biopesticide SolviNix® LC. http://news.agropages.com/News/NewsDetail---14600.htm. Accessed: June 5, 2015.

Anonymous (2015b) What are biopesticides? United States Environmental Protection Agency. http://www.epa.gov/pesticides/biopesticides/whatarebiopesticides.htm. Accessed: June 14, 2015.

Ash GJ (2010) The science, art and business of successful bioherbicides. Biol Control 52(3):230-240

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 K, & Mupondwa E (2006) Developing microbial weed control products: Commercialization, biological, and technological considerations. Pages 431-473 *in* Singh H, Batish D, Kohli R eds. Handbook of sustainable weed management. Binghamton, NY: The Haworth Press

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 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

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.

Boyette CD, Hoagland RE, Weaver MA (2008) Interaction of a bioherbicide and glyphosate for controlling hemp sesbania in glyphosate-resistant soybean. Weed Biol Manag 8(1):18-24

Boyette CD, Hoagland RE, Stetina KC (2014) Biological control of the weed hemp sesbania (*Sesbania exalta*) in rice (*Oryza sativa*) by the fungus *Myrothecium verrucaria*. Agronomy 4:74-89

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 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, Ontaro. http://www.guelphturfgrass.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.guelphturfgrass.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.guelphturfgrass.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.guelphturfgrass.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.guelphturfgrass.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

Charudattan R, Dinoor A (2000) Biological control of weeds using plant pathogens: accomplishments and limitations. Crop Prot 19(8-10):691-695

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

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

Ditmore M, Moore JW, TeBeest DO (2008) Interactions of two selected field isolates of *Colletotrichum gloeosporioides f. sp. aeschynomene* on *Aeschynomene virginica*. Biol Control 47(3):298-308

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

Duke SO, Dayan FE (2011) Modes of action of microbially-produced phytotoxins. Toxins 3(8):1038-1064

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

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

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

Graupner PR, Gerwick BC, Siddall TL, Carr AW, Clancy E, Gilbert JR, Derby JA (2006) Chlorosis inducing phytotoxic metabolites: New herbicides from *Phoma macrostoma*. Pages 37-47 *in* Rimando AM, Duke SO eds. Natural Products For Pest Management. Washington, USA: American Chemical Society

Grewal PS (1999) Factors in the success and failure of microbial control in turfgrass. Integrated Pest Manag Rev 4(4):287-294

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

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

Hoagland RE (1990) Microbes and microbial products as herbicides. An overview. ACS Sym Ser (439):2-52

Jarvis WR, Traquair JA, Bélanger RR (2007) Sporodex®, fungal biocontrol for powdery mildew in greenhouse crops. Pages 224-233 *in* Vincent C. Goettel MS, Lazarovits G eds. Biological control: a global perspective. Wallingford; UK: CABI

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. Accessed July 23, 2014

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

Kenney DS (1986) DeVine—the way it was developed–an industrialist's view. Weed Sci 34:15-16

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.

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

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

LeCong Z, Bailey KL, Derby J (2004) Plant colonization and environmental fate of the biocontrol fungus *Phoma macrostoma*. Biol Control 30(3):634-644

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: Eviron Sci Eng 49: 344-354

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Barrett M (2012) Reducing the risks of herbicide resistance: Best management practices and recommendations. Weed Sci 60:31-62

Ortiz-Ribbing L, Glassman KR, Roskamp GK, Hallett SG (2011) Performance of two bioherbicide fungi for waterhemp and pigweed control in pumpkin and soybean. Plant Dis 95(4):469-477

Pacanoski Z (2011) Bioherbicides - a real or virtual measure for efficient weed control? Herbologia 12(3): 103-114

Pitt WM, Bailey KL, YongBi F, Peterson GW (2012) Biological and genetic characterisation of *Phoma macrostoma* isolates with bioherbicidal activity. Biocontrol SciTechn 22(7):813-835

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

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

Sabba RP, Vaughn KC (1999) Herbicides that inhibit cellulose biosynthesis. Weed Sci 47:757-763

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

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

Timmons FL (2005) A history of weed control in the United States and Canada. Weed Sci 53(6): 748-761

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

Vencill WK, Nichols RL, Webster TM, Soteres JK, Mallory-Smith C, Burgos NR, Johnson WG, McClelland MR (2012) Herbicide resistance: Toward an understanding of resistance development and the impact of herbicide-resistant crops. Weed Sci 60:2-30

Webber CL, Taylor MJ, Shrefler JW (2014) Weed control in yellow squash using sequential postdirected applications of pelargonic acid. HortTechnology 24(1): 25-29

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

Zimdahl, R. (2013b) Weed science: A plea for thought - revisited. Fort Collins, CO, USA: Colorado State University. 73p

Selective Broadleaf Weed Control in Turf With the Bioherbicides *Phoma macrostoma* and Thaxtomin A

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

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 *Phoma macrostoma* and thaxtomin A on regionally important broadleaf weeds. In at least one of two years of container experiments, PRE applications of *Phoma 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 *Phoma macrostoma* and thaxtomin A peaked at 64% and 72% four weeks after treatment and declined afterwards,

---

[*] 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

suggesting that these bioherbicides possess short residuals and therefore must be re-applied for season-long control. Overall POST broadleaf weed control using *Phoma 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 ≥ 86%, henbit control, equal to pendimethalin. These results suggest that both *Phoma 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.

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 (Wilen 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 *Phoma macrostoma* and Thaxtomin A. *Phoma macrostoma*, a coelomycete fungal pathogen isolated from Canada thistle (*Cirsium arvense*), has been shown to cause bleaching, chlorosis, and eventual death in a variety of broadleaf weed species when applied both PRE and POST (Bailey et al. 2009; Evans et al. 2013). The fungus is nonpathogenic to monocots but has been shown to infect 38 economically important dicotyledonous weed species from 12 families (Bailey et al. 2011). Because of the pathogen's ability to control broadleaf weeds without harming grass species, commercial applications in the turfgrass industry are currently being explored; however, research on *Phoma 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 *Phoma macrostoma* (Neal et al. 2013). However, no data are currently available on *Phoma 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 *Phoma 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 *Phoma macrostoma* and thaxtomin A to control common weeds of turfgrass in the southern United States in container screenings, (2) determine product efficacy in newly-seeded turfgrass in field studies, and (3) evaluate the potential for enhancing the efficacy of thaxtomin A on poorly-controlled species with multiple applications and increased dose.

Materials and Methods

Common Methods. All experiments were conducted at the North Carolina State University Horticultural Field Lab in Raleigh, NC (35.79N, -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 m^2, 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 CO_2 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 (equal to the non-treated) and 10 = 100% control. Intermediate values are visual estimates of percent reduction in above ground plant biomass 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., 151 Gene E Stewart Blvd, Sylacauga, AL 35151) then hand watered to settle the substrate. Weeds were surface seeded 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 *Phoma macrostoma*. *Phoma 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-P_2O_5-K_2O) 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}, *Phoma 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 CO_2 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 CO_2-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}, *Phoma 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}, *Phoma 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, henbit was present in all plots and ranged from cotyledon to four leaf pair growth stages 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 *Phoma macrostoma* and thaxtomin A controlled some weed species equivalent to pendimethalin. For both *Phoma 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, *Phoma macrostoma* at 3250 and 6500 mu m^{-2} provided $\geq$ 90% control of marsh yellowcress and dandelion, equal to pendimethlin, but provided $\leq$ 58% control of all other species (Tables 1.1 & 1.2). At 13000 mu m^{-2}, *Phoma 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 1.1 & 1.2).

Weed control with *Phoma macrostoma* was lower in 2013 than in 2012. In 2013, *Phoma macrostoma* at 3250 and 6500 mu m^{-2} provided 32% and 58% control of dandelion, respectively, which was equal to pendimethalin, but provided $\leq$ 53% control of all other species (Tables 1.1 & 1.2). At 13000 mu m^{-2}, *Phoma 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 1.1 & 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 1.1 & 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 1.1 & 1.2). Thaxtomin A also provided 75% control of hairy galinsoga, equal to pendimethalin (Table

1.2). 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 1.1 & 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 bioherbicidal 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 treatments was dismissed as a causative factor. Application methods were identical between years. Temperatures over the course of the experiment were similar, averaging 21.7^{o} C in 2012 and 21.5^{o} 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 *Phoma 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 *Phoma 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). Weed counts are presented in Tables A.1.1. and A.1.2. 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 *Phoma macrostoma* and thaxtomin A doses increased. Injury symptoms caused by *Phoma 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 1.3). 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 1.3). At 13000 mu m^{-2}, *Phoma macrostoma* provided 64% control of henbit and 99% control of dandelion, but provided $\leq$ 28% control of common chickweed and field madder (Table 1.3). When evaluated 4WAT and 10WAT, overall broadleaf weeds were controlled 24 to27% 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 *Phoma macrostoma* (Table 1.3).

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 1.3). 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 1.3). 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 Phoma 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 1.3). 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, flexouous 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. Percent control three weeks after initial applications are presented in Tables A.1.3 and A.1.4.

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, *Phoma macrostoma* was not effective when applied POST, controlling all species < 54% in both years regardless of application rate, and no *Phoma macrostoma* treatment controlled any species in the experiment equal to the auxinic herbicide (Tables 1.4 and 1.5).

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 1.4). 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 ≤ 28% control of marsh yellowcress, flexuous bittercress, dandelion, and common chickweed, none of which were equal to the auxinic herbicide (Table 1.5). In the 2013 experiment, control of henbit, marsh yellowcress, and flexuous bittercress with thaxtomin A at 190 g ai ha^{-1} was ≥ 63%, but was not equal to the auxinic herbicide. Dandelion and common chickweed were controlled 22% and 28%, respectively (Table 1.4).

In both years, thaxtomin A at 380 g ai ha^{-1} provided ≥ 80% control of yellow woodsorrel, hairy galinsoga and ivyleaf speedwell, equal to or better than the auxinic herbicide (Table 1.4). 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 ≤ 74% in 2012, not equal to the auxinic herbicide (Table 1.5).

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 1.5).

Plants typically displayed some chlorosis or bleaching following applications of *Phoma macrostoma*, but recovery was rapid following application. Complete bleaching and necrosis were not observed on any species after applications of *Phoma 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 *Phoma 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 *Phoma macrostoma*. These differences may be related to differences in product formulation and application rates, as the rate of *Phoma 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 *Phoma macrostoma* provided $\leq$ 10% control of common chickweed in both years when applied POST (Table 1.6). 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 *Phoma macrostoma* controlled sparrow vetch equal to the auxinic herbicide (Table 1.6). 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. *Phoma macrostoma* provided about 25% control of henbit, equal to the auxinic herbicide (Table 1.6). 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 1.6). Further percent control and percent cover evaluations collected 24 weeks after treatment are presented in Table A.1.5. 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. Additional data from these experiments is presented in Tables A.1.6., A.1.7., and A.1.8.

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 1.7). 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 1.7). 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 1.8). 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 1.8). 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 *Phoma 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.

Acknowledgements

The authors express appreciation to the USDA IR-4 Biopesticides Program, The Scotts Co. and Marrone Bio Innovations for funding this research.

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 1.1. 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).

			Yellow woodsorrel		Henbit		Marsh yellowcress		Flexuous bittercress	
Herbicide	Rate	Rate Unit[a]	2012	2013	2012	2013	2012	2013	2012	2013
			-----------------------------------% Control[b]---							
P. macrostoma	3250	mu m^{-2}	10	15	3	17	90	37	36	27
P. macrostoma	6500	mu m^{-2}	8	15	20	7	100	53	58	25
P. macrostoma	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 1.2. 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]--							
P. macrostoma	3250	mu m^{-2}	6	13	90	32	4	18	26	10
P. macrostoma	6500	mu m^{-2}	16	5	100	58	10	7	12	5
P. macrostoma	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 1.3. 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).

			Weed control[a], 4 WAT[b]				Combined Broadleaf Weeds[c]			Tall
Herbicide	Rate	Rate Unit	Henbit	Dandelion	Common chickweed	Field madder	4 WAT	10 WAT	32 WAT	Fescue[d], 10 WAT
			---------------------------% Control-------------------------------------							-% Cover-
P. macrostoma	6500	mu m^{-2}	19	87	13	12	27	24	50	72
P. macrostoma	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.

Table 1.3 Continued

[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 1.4. 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	Rate Unit	Yellow woodsorrel	Hairy galinsoga	Ivyleaf speedwell
			--------------------% Control[b]--------------------		
P. macrostoma	3250	mu m^{-2}	20	5	0
P. macrostoma	6500	mu m^{-2}	13	10	3
P. macrostoma	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] 0.026% dicamba, 0.605% 2,4-dichlorophenoxyacetic acid, 0.149% methylchlorophenoxypropionic acid

Table 1.5. 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).

			Henbit[a]		Marsh yellowcress		Flexuous bittercress		Dandelion		Common chickweed	
Herbicide	Rate[a]	Rate Unit	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
			% Control[b]									
P. macrostoma	3250	mu m^{-2}	0	5	16	18	2	10	10	3	2	2
P. macrostoma	6500	mu m^{-2}	0	5	42	10	16	14	20	7	0	5
P. macrostoma	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] 0.026% dicamba, 0.605% 2,4-dichlorophenoxyacetic acid, 0.149% methylchlorophenoxypropionic acid

Table 1.6. 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).

			Common chickweed		Sparrow vetch				
Herbicide[a]	Rate	Rate Unit	2012	2013	2012	2013	Henbit[b]	Dandelion	Broadleaf weeds[c]
			---% Control[d]---						
P. macrostoma	6500	mu m^{-2}	5	10	28	53	24	40	29
P. macrostoma	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.

Table 1.6 Continued

[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] 0.026% dicamba, 0.605% 2,4-dichlorophenoxyacetic acid, 0.149% methylchlorophenoxypropionic acid

Table 1.7. POST control of henbit using thaxtomin A. Research conducted at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

		Henbit Control[ab]		
Herbicide	Rate	4WAT[c]	10WAT	16WAT
	g ai ha^{-1}	--------------------%---------------------		
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 1.8. 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).

		Henbit Control[ab]		
Herbicide	Rate	4WAT[c]	4WAT2	16WAT2
	g ai ha^{-1}	--------------------%[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.

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

Joseph C. Wolfe, Joseph C. Neal, Christopher D. Harlow, and Travis W. Gannon[*]

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. When applied at 380 g ai ha^{-1}, thaxtomin A reduced tall fescue cover when applied at least 1 week before seeding (WBS), at seeding (AS), and 1 week after seeding (WAS), but was safe at other application timings,

---

[*] First, second and third authors: graduate student, professor, and research technician, respectively, Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609; fourth author: assistant professor, Department of Crop Science, North Carolina State University, Raleigh, NC 276950-7609. Corresponding author’s email: jcwolfe@ncsu.edu

while applications at 570 g ai ha^{-1} also reduced tall fescue cover when applied 2 WBS and 2 WAS. Both rates of thaxtomin A reduced 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. Furthermore, these results suggest that applications of thaxtomin A at 380 g ai ha^{-1} will not injure tall fescue or perennial ryegrass when applied at least 2 WBS or 2 WAS, and that thaxtomin A may provide effective PRE control of annual bluegrass while also allowing the safe establishment of perennial ryegrass by making applications 6 WBS, 2 WBS, and 2 WAS.

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.

Key words: biological weed control; biopesticide; seeding intervals.

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 (Bourdot 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 theses 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 *Dreschslera 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 could provide 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 thaxtomin A's ability 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 does have PRE activity on grasses, thaxtomin A did not reduce tall fescue cover when applied four weeks after seeding, suggesting that it may potentially be used for this purpose (Chapter 1).

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.

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 m^2 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 2.1. 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.

Treated plots in these experiments were 1 by 2 m in size. All treatments were applied with a CO_2 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 m^2 in size in 2014. All treatments were applied with a CO_2 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 kill 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 CO_2 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/seeder 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 ($N-P_2O_5-K_2O$) 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 ($N-P_2O_5-K_2O$) 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, 4 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 1 meter by 1 meter 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-P_2O_5-K_2O) 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-P_2O_5-K_2O) 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 evauate the safety of thaxtomin A when applied to creeping bentgrass (*Agrostis stolonifera*, 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 0.6cm 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 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 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. Percent cover data are provided in Table A.2.1.

Corn gluten meal provided 0% control of smooth crabgrass at all rating dates, and resulted in equal or greater crabgrass cover than non-treated plots (Table A.2.1). 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 2.1). 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 2.1). 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 2.1).

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 2.1). 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 equal 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 2.1).

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). Percent cover data are presented in Table A.2.2.

Corn gluten meal provided 0% control of annual bluegrass at all rating dates, and resulted in equal or greater annual bluegrass cover than non-treated plots (Table A.2.2). 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 2.2). 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 2.2). 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 2.2).

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 2.2). 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 2.2).

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). See Table A.2.3. for fresh weight data. 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 2.3). 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 2.3). 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 2.3). 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 2.3).

When applied at 190 g ai ha^{-1}, thaxtomin A caused $\leq$ 1% injury to perennial ryegrass regardless of timing (Table 2.3). 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 2.3). 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 2.3).

Tall Fescue Safety. Treatment-by-location interactions were non-significant at all evaluation dates, thus data was 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 4 WBS, neither rate of thaxtomin A reduced tall fescue cover compared to non-treated turf. When applied 2 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 2.4). Thaxtomin A at 380 g ai ha^{-1} did not reduce tall fescue cover when applied 2 WBS. Applied 1 WBS, AS and 1 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 2.4). When applied 2

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 2.4). Thaxtomin A at 380 g ai ha^{-1} applied 2 WAS did not cause injury. When applied 4 and 6 WAS, neither rate of thaxtomin A significantly reduced tall fescue cover (Table 2.4). 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 2 WBS, and that applications made 2 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 4 WBS and 4 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 4 WAS, neither dose of thaxtomin A applied 4 WBS reduced perennial ryegrass cover. When applied 2 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 1 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 2.5). Applications of thaxtomin A made 1 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 2 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 2.5).

At the final evaluation 10 WAS, the only treatments which reduced perennial ryegrass cover at either location were both rates of thaxtomin A applied 1 WBS, AS, and 1 WAS. Perennial ryegrass cover was reduced by thaxtomin A at 380 g ai ha^{-1} applied 1 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 1 WAS reduced perennial ryegrass cover at both sites. At 570 g ai ha^{-1}, thaxtomin A reduced perennial ryegrass when applied 1 WBS, AS, and 1 WAS. Perennial ryegrass cover was unaffected by the sequential applications of 380 g ai ha^{-1} thaxtomin A made 2 WBS and 2 WAS, or by three applications made 2 WBS, 2 WAS, and 6 WAS (Table 2.4).

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 2.6). Thaxtomin A at 190 g ai ha^{-1} caused 20% injury to creeping bentgrass when evaluated 4 WAT, though the severity of injury had decreased to 13% by 16 WAT

(Table 2.6). When applied at 380 g ai ha-1, thaxtomin A injured creeping bentgrass 38% 4 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 2.6). 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 2.6). 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 2.6). 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 2.6).

These data suggest that 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 2.1). Creeping bentgrass mowed at greens height 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.

Acknowledgements

The authors express appreciation to the USDA IR-4 Biopesticides Program and Marrone Bio Innovations for funding this research.

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

					Percent control of smooth crabgrass							
	Rate at each application[a]				June		July		August		September	
Herbicide	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

Table 2.1 Continued

$LSD_{0.05}$	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 2.2. PRE control of annual bluegrass with thaxtomin A and pendimethalin. Research conducted at Thorndale Country Club (Oxford, NC).

	Rate at each application[a]			Percent control of annual bluegrass[b]					
				November		January			
Herbicide	1	2	3	2013	2014	2013	2014	March	April
	----- 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 2.3. 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).

		Injury	
Timing	Rate	FESAR	LOLPE
	g ai ha^{-1}	----------%	-------------
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

Table 2.3 Continued

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

Table 2.4. 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).

		Tall fescue cover^{ab}				
Timing	Rate	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

Table 2.4 Continued

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 2.5. 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).

		Percent cover of perennial ryegrass[a]			
		4WAS		10WAS	
Timing	Rate	LW	SH	LW	SH
	g ai ha^{-1}	-------------------------%	------------------------------		
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

Table 2.5 Continued

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.

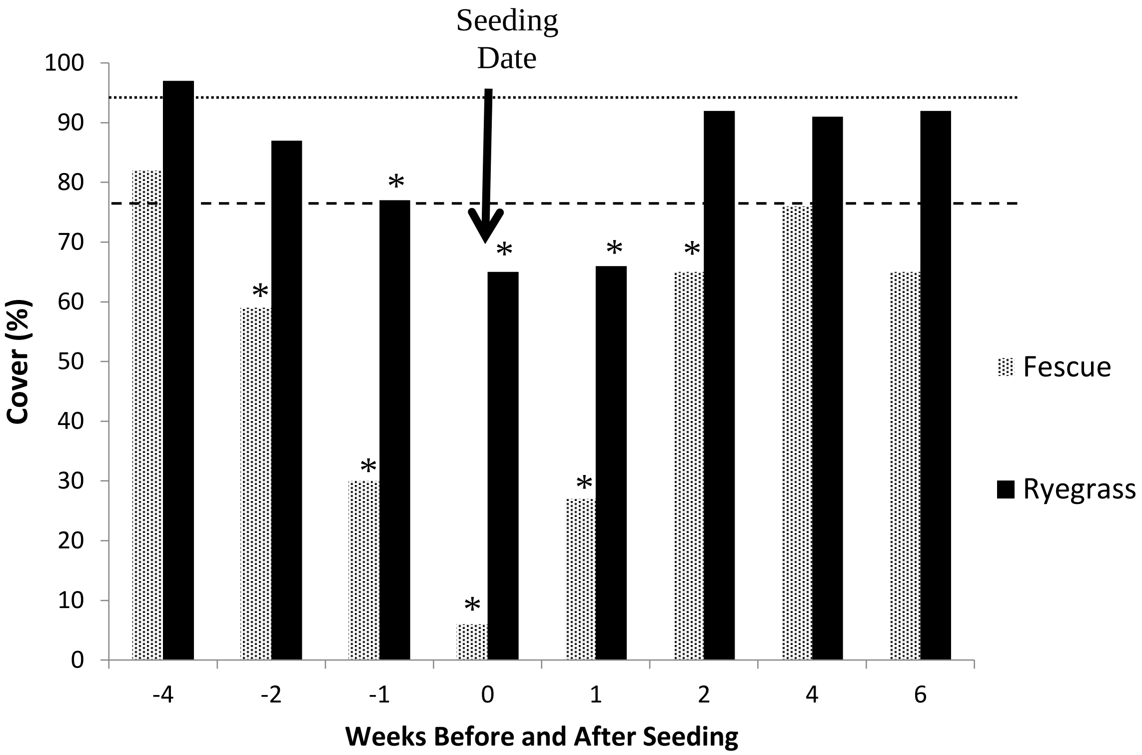

Figure 2.1. Effects of thaxtomin A at 570 g ai ha^{-1} applied pre- and post-seeding on percent cover of newly seeded tall fescue and over-seeded perennial ryegrass. Data are from visual estimates of turfgrass cover evaluated 10 weeks after seeding. Dashed line represents average percent cover of tall 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 2.6. Effect of applications of thaxtomin A on creeping bentgrass. Research conducted at the North Carolina State University Lake Wheeler Field Laboratory (Raleigh, NC).

	Dose at each application		Percent creeping bentgrass injury			
Treatment	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 .

Postemergence Broadleaf Weed Control with the Bioherbicide FeHEDTA

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

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

---

[*] 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

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.

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.

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 a large number of annual and perennial broadleaf weeds, causing foliar necrosis in target species within three days of application without injuring turf (Wilen 2012). While its mechanism of action and selectivity is not yet fully understood, it is possible that iron absorbed as FeHEDTA can function 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 lupuplina*) 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, FeHEDTA has only been tested on three (Webster 2012). 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}, re-applied 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, -78.7W). 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 m^2 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 CO_2 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 Laker 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 m^2 grid count to record white clover frequency. A 1 m^2 frame was divided into 100 equal sections of 1 dm^2 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 ($N-P_2O_5-K_2O$) 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 CO_2 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-P_2O_5-K_2O) 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 CO_2 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

CO_2-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 CO_2 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 dm^2 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 was further subjected to nonlinear regression analysis over FeHEDTA dose using the four-parameter log-logistic model described in Equation 3.1 (Price et al. 2012):

$$Y = C + (D - C) / \{1 + \exp[B(\log X - \log E)]\} \quad [3.1]$$

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 3.1). 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 both main effects of spray volume and concentration were significant, but the interaction between these factors was not (Table 3.1). 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 3.2). 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 3.2).

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 3.2). 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 3.1). When these data were plotted, the resulting means fit a non-linear dose response curve (Figure 3.1).

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 3.2). 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 3.3). 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 3.3). 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 3.5). 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 3.5). 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 3.5).

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 3.6). 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 3.6). 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, but 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 3.7). 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 3.7). 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 3.7). 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 compare 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, 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.

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

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

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 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, Ontaro. http://www.guelphturfgrass.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.guelphturfgrass.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.guelphturfgrass.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.guelphturfgrass.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.guelphturfgrass.ca/sites/default/files/research_reports/2012-Tolerance%20of%20new%20seedings%20of%20different%20grass%20species%20t o%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. 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: Eviron 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

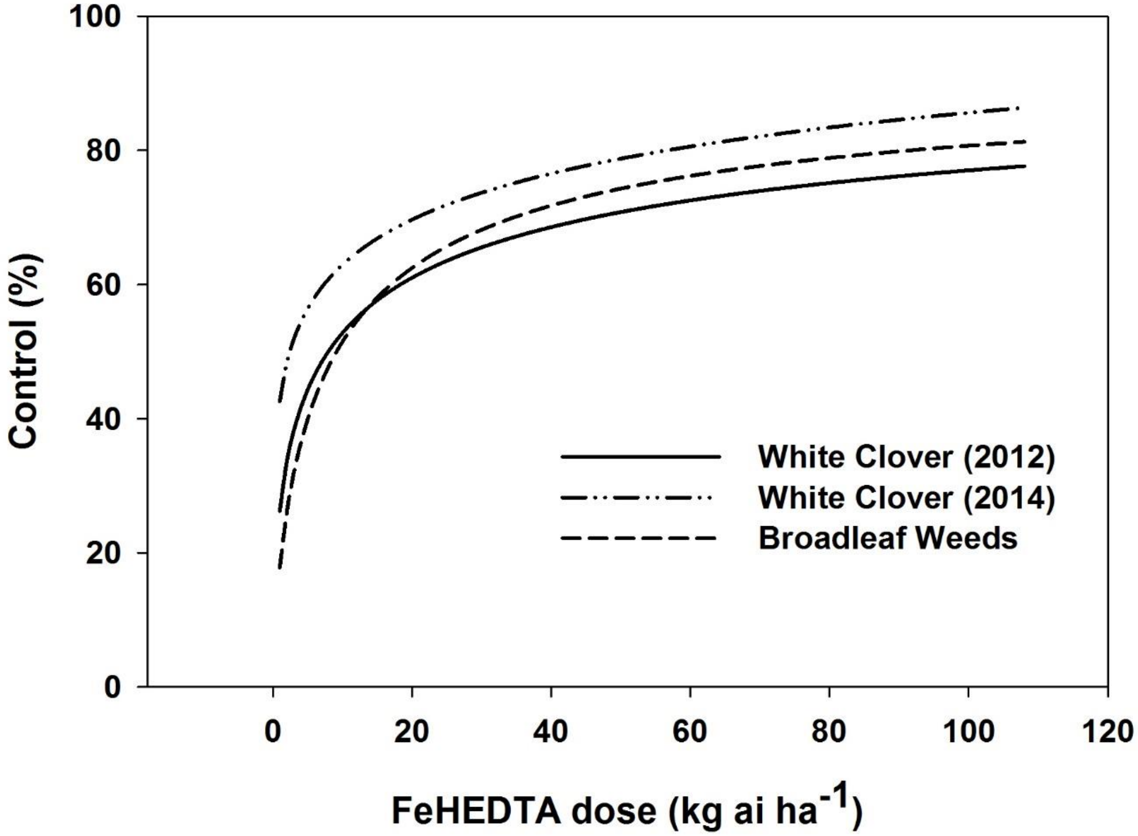

Figure 3.1. Effect of FeHEDTA dose on control (%) of white clover and winter annual broadleaf weeds; evaluated six weeks after second application on white clover and four weeks after second application on winter annual broadleaf weeds (6WAT2 and 4WAT2, respectively). The regression lines were plotted using Equation 3.1, and the parameter values are presented in Table 3.4.

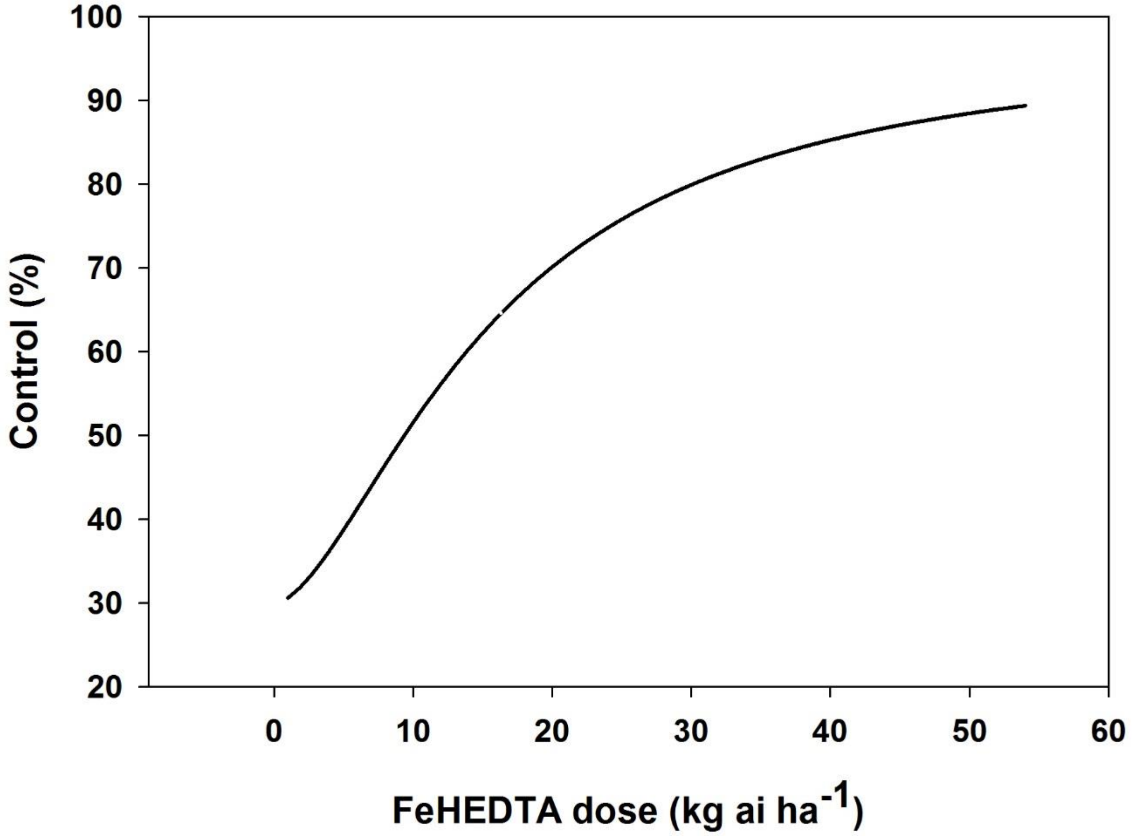

Figure 3.2. Effect of FeHEDTA dose on control (%) of white clover, six weeks after second application (6WAT2). The regression line was plotted using Equation 3.1, and the parameter values are presented in Table 3.4.

Table 3.1. 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).

				6WAT2		3WAT1		
Concentration	Volume	Dose	3WAT1[a,b]	2012	2014	2012	2014	6WAT2
(v/v)	(L ha^{-1})	(kg ai ha^{-1})	--------------% Control[c]--------------			----------------% Cover---------------		
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

Table 3.1 Continued

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 3.2. 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).

					TAROF[c]			
			WABRDLF[ab]		3WAT1		6WAT2	
Concentration	Volume	Dose	3WAT1	4WAT2	2012	2014	2012	2014
(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

Table 3.2 Continued

$LSD_{0.05}$	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 3.3. 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 3.4. Model parameters and standard errors for the four-parameter log-logistic model (provided in Equation 3.1) for Figures 3.1 and 3.2 for white clover and winter annual broadleaf weed response to two POST applications of FeHEDTA.

	Model Parameters (± SE)			
Experiment	*B*	*C*	*D*	*E*
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 3.5. 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).

							LAMAM[c]		CARFL	
Herbicide	Dose	TAROF[ab]	OXAST	RORIS	VERHE	STEME	2012	2013	2012	2013
	kg ai ha^{-1}	---------------------------------------	----% 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 3.6. 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).

		TAROF[ab]				
Herbicide	Dose	2DAT	4WAT	VICTE	TRFCA	STEME
	kg ai ha^{-1}	-----------------------------% 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 3.7. 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).

		LAMAM[a][b]		STEME		LAMAM		STEME	
Dose	Interval	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.

Table 3.7 Continued

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

APPENDICES

Appendix A. Selective Broadleaf Weed Control in Turf With the Bioherbicides *Phoma macrostoma* and Thaxtomin A.

Table A.1. Weed counts of broadleaf weeds following PRE applications of bioherbicides. Research conducted in 2012 at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

			Weed counts, 6 WAT[a]					
Herbicide[a]	Rate	Rate Unit	Henbit	Dandelion	Common chickweed	Field madder	Large hop clover	Carolina geranium
Non-treated	NA	NA	2	22	5	6	34	4
P. macrostoma	6500	mu m^{-2}	4	1	6	6	13	4
P. macrostoma	13000	mu m^{-2}	1	0	4	15	3	3
Thaxtomin A	190	g ai ha^{-1}	5	4	4	9	8	3
Thaxtomin A	380	g ai ha^{-1}	1	1	3	9	3	2
Corn Gluten	97	kg ha^{-1}	3	10	5	8	9	4
$LSD_{0.05}$			4	8	4	16	8	3

[a] Abbreviations: *P. macrostoma*, *Phoma macrostoma*; mu, macrocidin unit; WAT, weeks after treatment; NA, not applicable.

Table A.2. Weed counts of broadleaf weeds following PRE applications of bioherbicides. Research conducted in 2013 at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

			Weed counts, 6 WAT[a]							
Herbicide[a]	Rate	Rate Unit	Henbit	Dand-elion	Common chick-weed	Field madder	Mouse-ear chic-kweed	Carolina geranium	Yellow woods-orrel	Sparrow vetch
Non-treated	NA	NA	24	8	3	64	53	14	28	35
P. macrostoma	6500	mu m^{-2}	18	1	6	83	32	14	14	45
P. macrostoma	13000	mu m^{-2}	9	0	12	43	9	17	12	23
Thaxtomin A	190	g ai ha^{-1}	5	8	3	40	12	18	18	48
Thaxtomin A	380	g ai ha^{-1}	3	6	8	37	4	19	14	62
Corn Gluten	97	kg ha^{-1}	33	6	19	43	29	14	16	67
$LSD_{0.05}$			15	4	7	25	29	6	13	26

[a] Abbreviations: *P. macrostoma*, *Phoma macrostoma*; mu, macrocidin unit; WAT, weeks after treatment; NA, not applicable.

Table A.3. Postemergence control of container-grown yellow woodsorrel, hairy galinsoga, and ivyleaf speedwell using bioherbicides, 21 days after the first application. Research conducted at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

Herbicide[a]	Rate	Rate Unit	Yellow woodsorrel	Hairy galinsoga	Ivyleaf speedwell
			--------------------% Control[b]	--------------------	
P. macrostoma	3250	mu m^{-2}	10	5	5
P. macrostoma	6500	mu m^{-2}	9	8	4
P. macrostoma	13000	mu m^{-2}	15	11	5
Thaxtomin A	190	g ai ha^{-1}	58	45	38
Thaxtomin A	380	g ai ha^{-1}	79	64	55
Weed B Gon[c]	412	g ai ha^{-1}	87	79	48
$LSD_{0.05}$			14	11	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. Data pooled across years as treatment-by-year effect was not significant.

[c] 0.026% dicamba, 0.605% 2,4-dichlorophenoxyacetic acid, 0.149% methylchlorophenoxypropionic acid

Table A.4. Postemergence control of container-grown henbit, marsh yellowcress, flexuous bittercress, dandelion and common chickweed using bioherbicides 21 days after the first application. Research conducted at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

Herbicide	Rate	Rate Unit	Henbit 2012	Henbit 2013	Marsh yellowcress 2012	Marsh yellowcress 2013	Flexuous bittercress 2012	Flexuous bittercress 2013	Dandelion 2012	Dandelion 2013	Common chickweed 2012	Common chickweed 2013
			% Control[b]									
P. macrostoma[a]	3250	mu m^{-2}	0	2	16	5	6	8	12	18	2	0
P. macrostoma	6500	mu m^{-2}	4	5	10	10	2	18	16	20	0	3
P. macrostoma	13000	mu m^{-2}	8	8	16	22	16	15	24	25	0	10
Thaxtomin A	190	g ai ha^{-1}	42	35	26	50	30	48	36	25	12	30
Thaxtomin A	380	g ai ha^{-1}	64	58	70	82	62	78	48	55	20	57
Weed B Gon[c]	412	g ai ha^{-1}	22	52	96	100	92	93	36	87	66	68
$LSD_{0.05}$			12	12	17	7	18	13	17	12	10	8

[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 = complete plant mortality.

[c] 0.026% dicamba, 0.605% 2,4-dichlorophenoxyacetic acid, 0.149% methylchlorophenoxypropionic acid

Table A.5. Percent control of dandelion and percent control and cover of mixed winter annual broadleaf weeds in tall fescue turf twenty-four weeks after applications of FeHEDTA and Weed B Gon. Research conducted at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

			Overall broadleaf weeds[b]			
Herbicide	Rate	Rate Unit	2012	2013	2012	2013
			---% Control[c]---		---% Cover ---	
P. macrostoma[a]	6500	mu m^{-2}	42	15	21	62
P. macrostoma	13000	mu m^{-2}	68	20	6	57
Thaxtomin A	190	g ai ha^{-1}	37	13	20	70
Thaxtomin A	380	g ai ha^{-1}	17	8	32	70
Weed B Gon[d]	412	g ai ha-1	92	88	3	14
$LSD_{0.05}$			37	13	6	21

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

[b] Overall broadleaf weeds includes Persian speedwell, ivyleaf speedwell, common chickweed, mouseear chickweed and field madder.

[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 A.6. Henbit cover following POST applications of the bioherbicide thaxtomin A. Research conducted in 2014 at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

		Henbit cover		
Herbicide	Rate	4WAT[a]	10WAT	16WAT
	g ai ha^{-1}	------------------%	-------------------	
Non-treated	NA	45	29	25
Thaxtomin A	190	32	16	16
Thaxtomin A	380	24	7	11
Thaxtomin A	570	11	5	5
$LSD_{0.05}$		13	5	5

[a] Abbreviations: WAT, weeks after treatment; NA, not applicable.

Table A.7. Henbit cover following PRE- followed by early POST applications of thaxtomin A. Research conducted at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

		Henbit cover[a]		
Herbicide	Rate	4WAT[b]	4WAT2	16WAT2
	g ai ha^{-1}	--------------------%---------------------		
Non-treated	NA	64	71	28
Thaxtomin A	190	23	43	11
Thaxtomin A	380	25	9	3
Pendimethalin	3300	1	2	0
LSD$_{0.05}$		13	7	9

[a] Data pooled across years as treatment-by-year effect was not significant.

[b] Abbreviations: WAT, weeks after treatment; WAT2, weeks after second treatment; NA, not applicable.

Table A.8. Control of common chickweed after PRE- followed by early POST applications of thaxtomin A. Research conducted in 2014 at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

Herbicide	Rate	Common chickweed control[a] 4WAT[c]	4WAT2	16WAT2
	g ai ha^{-1}	--------------------%[c]	---------------------	
Thaxtomin A	190	17	62	37
Thaxtomin A	380	40	94	81
Pendimethalin	3300	98	97	74
$LSD_{0.05}$		17	4	46

[a] Data pooled across years as treatment-by-year effect was not significant.

[b] Abbreviations: WAT, weeks after treatment; WAT2, weeks after second treatment; NA, not applicable.

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

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

Table B.1. Smooth crabgrass cover following PRE applications of thaxtomin A and pendimethalin. Research conducted at Thorndale Country Club (Oxford, NC).

					Percent cover of smooth crabgrass							
	Rate at each application[a]				June		July		August		September	
Herbicide	1	2	3	4	2013	2014	2013	2014	2013	2014	2013	2014
	--------- g ai ha^{-1} ----------				---------------------------------% ---------------------------------------							
					80	67	98	74	98	74	------	81
Thaxtomin A	190	0	0	0	80	57	100	62	100	74	------	80
Thaxtomin A	380	0	0	0	50	60	70	75	82	77	------	86
Thaxtomin A	570	0	0	0	37	60	55	70	67	75	------	83
Thaxtomin A	190	190	0	0	47	40	60	53	63	73	------	77
Thaxtomin A	380	190	0	0	22	37	38	57	50	65	------	80
Thaxtomin A	380	380	0	0	14	39	16	52	36	67	------	71
Thaxtomin A	190	190	190	0	25	50	45	57	60	83	------	87
Thaxtomin A	380	190	190	0	------	45	------	60	------	82	------	80
Thaxtomin A	380	380	380	0	------	24	------	40	------	57	------	67
Thaxtomin A	190	190	190	190	------	44	------	45	------	52	------	80
Thaxtomin A	380	190	190	190	------	35	------	60	------	67	------	82
Thaxtomin A	380	380	380	380	------	17	------	27	------	45	------	53

Table B.1 Continued

Corn gluten	96000		96000		100	------	100	------	100	------	------	------
Pendimethalin	1680	0	1680	0	7	15	10	19	4	31	------	32
LSD$_{0.05}$					20	13	25	20	21	18	------	16

---

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

Table B.2. Percent cover of annual bluegrass following PRE applications of thaxtomin A and pendimethalin. Research conducted at Thorndale Country Club (Oxford, NC).

	Rate at each application[a]			Percent cover of annual bluegrass							
				November		January		March		April	
Herbicide	1	2	3	2013	2014	2013	2014	2013	2014	2013	2014
	----- g ai ha^{-1} -----			---% ---							
Non-treated	NA	NA	NA	22	32	62	52	67	57	75	62
Thaxtomin A	190	0	0	9	31	41	50	49	55	62	77
Thaxtomin A	380	0	0	4	30	14	60	25	57	37	67
Thaxtomin A	190	190	0	12	11	46	32	47	37	72	57
Thaxtomin A	380	190	0	8	10	30	19	35	27	45	53
Thaxtomin A	380	380	0	5	6	17	22	19	27	35	65
Thaxtomin A	190	190	190	6	24	40	55	37	55	52	80
Thaxtomin A	380	190	190	4	7	10	17	14	22	15	33
Thaxtomin A	380	380	380	4	6	10	17	12	18	16	35
Corn gluten	96000	0	0	34	------	76	------	77	-------	99	-------
Pendimethalin	1680	0	0	2	9	4	22	14	23	14	40
$LSD_{0.05}$				12	11	24	20	19	19	20	21

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

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

		Fresh weight (g)	
Timing	Rate	FESAR[a]	LOLPE
	g ai ha^{-1}		
AS	0	1.34	2.38
AS	190	0.72	2.21
AS	380	0.44	1.75
AS	570	0.18	1.72
1 WAS	0	1.00	2.52
1 WAS	190	0.90	1.91
1 WAS	380	0.35	2.05
1 WAS	570	0.38	1.77
2 WAS	0	0.95	1.31
2 WAS	190	0.89	1.25
2 WAS	380	0.54	1.23
2 WAS	570	0.44	1.13
4 WAS	0	0.72	1.91
4 WAS	190	0.56	1.41
4 WAS	380	0.55	1.77
4 WAS	570	0.41	1.88
6 WAS	0	0.48	1.59
6 WAS	190	0.59	1.95
6 WAS	380	0.41	1.60
6 WAS	570	0.59	1.67
$LSD_{0.05}$		0.30	0.51
ANOVA P values			

Table B.3 Continued

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.

Appendix C. Postemergence Broadleaf Weed Control with the Bioherbicide FeHEDTA

Table C.1. Percent cover of mixed winter annual broadleaf weeds 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).

			WABRDLF[ab]	
Concentration	Volume	Dose	3WAT1	4WAT2
(v/v)	(L ha^{-1})	(kg ai ha^{-1})	------------%	------------
1.95	467	3.375	No data	91
1.95	935	6.75	No data	85
1.95	1870	13.5	No data	55
1.95	3741	27	No data	30
3.9	467	6.75	No data	31
3.9	935	13.5	No data	66
3.9	1870	27	No data	36
3.9	3741	54	No data	20
7.8	467	13.5	No data	9
7.8	935	27	No data	29
7.8	1870	54	No data	22
7.8	3741	108	No data	3
$LSD_{0.05}$				21
ANOVA P values				
Volume			NA	<0.0001
Concentration			NA	<0.0001
Volume X Concentration			NA	---NS---

[a] Abbreviations: WABRDLF, winter annual broadleaf weeds; 3WAT1, 3 weeks after treatment 1; 4WAT2, 4 weeks after treatment 2; NA, not applicable; NS, not significant.

[b] Mixed winter annual broadleaf weeds includes Persian speedwell, ivyleaf speedwell,

Table C.1 Continued

common chickweed, and field madder.

Table C.2. Percent control of container-grown broadleaf weeds three weeks after one application of FeHEDTA and Weed B Gon. Research conducted in outdoor containers at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

						TAROF		STEME		LAMAM	
Herbicide	Dose	OXAST[ab]	RORIS	VERHE	CARFL	2012	2013	2012	2013	2012	2013
	kg ai ha^{-1}	---% Control[c]--									
FeHEDTA	29.7	66	76	84	32	70	88	4	0	26	3
FeHEDTA	59.4	83	85	85	44	93	95	10	5	34	13
Weed B Gon[d]	0.412	87	98	49	93	36	87	66	90	22	52
$LSD_{0.05}$		14	6	9	14	29	7	13	4	19	9

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

[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.

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

Table C.3. Percent control of dandelion and percent control and cover of mixed winter annual broadleaf weeds in tall fescue turf twenty-four weeks after applications of FeHEDTA and Weed B Gon. Research conducted at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

			Overall broadleaf weeds[b]			
Herbicide	Dose	Dandelion[a]	2012	2013	2012	2013
	kg ai ha^{-1}	------------% Control[c]-----------			----% Cover----	
FeHEDTA	29.7	92	70	23	7	55
FeHEDTA	59.4	97	77	25	8	55
Weed B Gon[d]	0.412	91	92	88	3	14
$LSD_{0.05}$		13	12	31	6	21

[a] Data pooled across years as treatment-by-year effect was not significant.

[b] Overall broadleaf weeds includes Persian speedwell, ivyleaf speedwell, common chickweed, mouseear chickweed and field madder.

[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

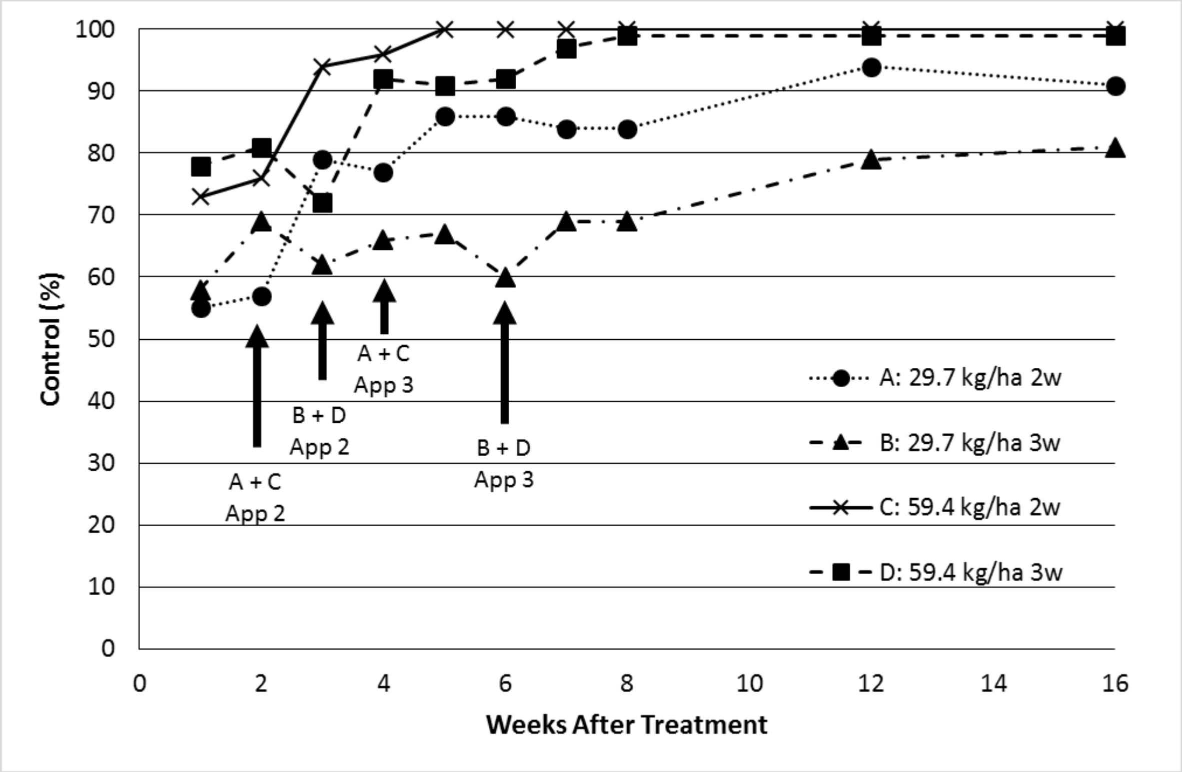

Figure C.1. Effects of FeHEDTA applied at two and three week intervals on percent control of henbit. Data are from visual estimates of percent control based on above-ground reductions in plant biomass. Arrows denote dates on which treatments were re-applied. Experiment conducted in 2013 at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).

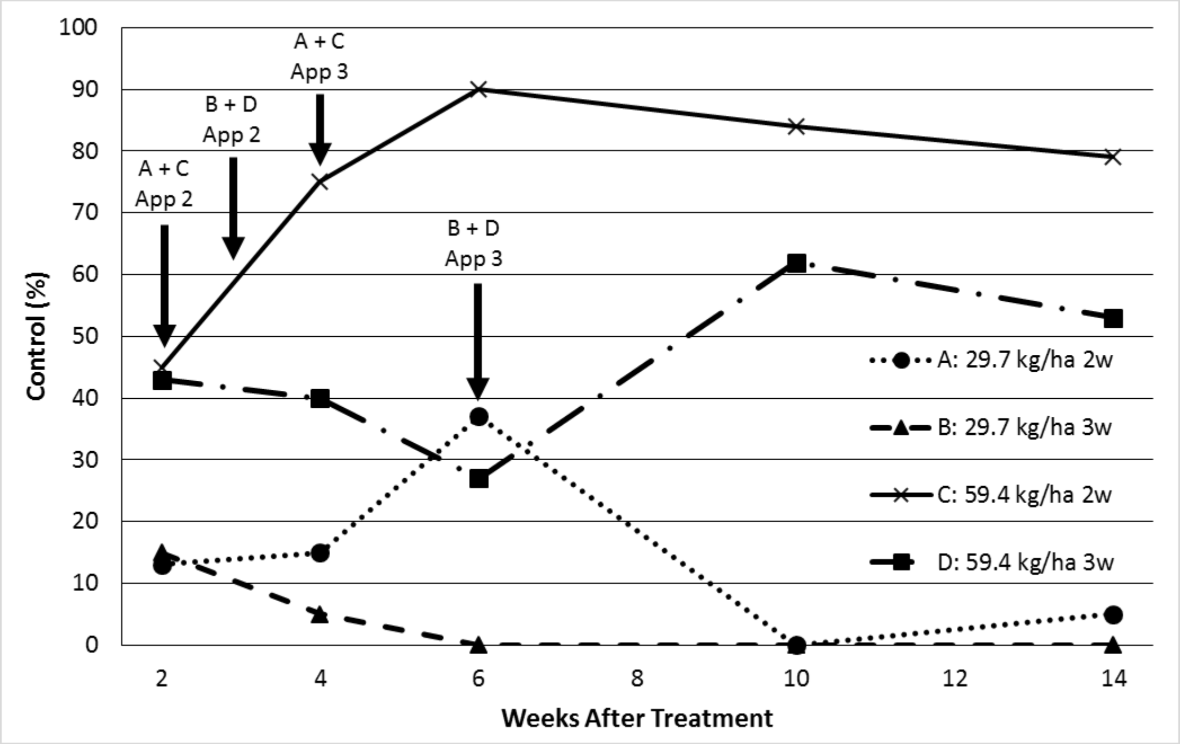

Figure C.2. Effects of FeHEDTA applied at two and three week intervals on percent control of common chickweed. Data are from visual estimates of percent control based on above-ground reductions in plant biomass. Arrows denote dates on which treatments were re-applied. Experiment conducted in 2014 at the North Carolina State University Horticultural Field Laboratory (Raleigh, NC).