

**Interaction of Methiozolin with Soil and Subsequent Influence on *Poa annua* Control**

by

Michael Luke Flessner

A dissertation submitted to the Graduate Faculty of  
Auburn University  
in partial fulfillment of the  
requirements for the Degree of  
Doctor of Philosophy

Auburn, Alabama  
August 2, 2014

Key words: desorption, retention, sorption, translocation

Copyright 2014 by Michael L. Flessner

Approved by

J. Scott McElroy, Co-chair, Associate Professor of Crop, Soil and Environmental Sciences  
Glenn R. Wehtje, Co-chair, Professor of Crop, Soil and Environmental Sciences  
Julie A. Howe, Associate Professor of Crop, Soil and Environmental Sciences  
James H. Baird, Assistant Specialist in Cooperative Extension and Assistant Horticulturist

## Abstract

Annual bluegrass is a problematic turfgrass weed due to its ability to thrive at low mowing heights, disruption of aesthetics and utility, and few herbicidal control options. Methiozolin is a new, currently unregistered herbicide that selectively controls annual bluegrass in desirable turfgrasses. Studies were conducted to evaluate and compare annual bluegrass control from preemergence (PRE) applied methiozolin as influenced by rate and soil type and from postemergence (POST) applied methiozolin as influenced by rate, soil type, annual bluegrass growth stage, and treatment placement. Studies were also conducted to evaluate foliar and root absorption and subsequent translocation of methiozolin by annual bluegrass using radio-tracer techniques. PRE applied methiozolin controlled annual bluegrass > 99%. POST applied methiozolin resulted in < 80% control regardless of foliar versus root exposure. POST applications are more effective at higher rates and smaller growth stages. Foliar-plus-soil methiozolin application trended to result in the best control, compared to foliar-only or soil-only applications. Absorption and translocation data indicate that methiozolin is absorbed by both leaves and roots and moderately translocates upward in the plant toward the leaf tip with little to no basipetal translocation. Due to the fact that control is limited from a single methiozolin application (as observed in POST experiments), successful field application of methiozolin requires multiple, timely applications directed toward the roots and/or foliage of annual bluegrass.

Previous research indicates that maximum weed control efficacy requires root exposure; however, soil sorption and mobility of methiozolin has not been established. Research was

conducted to investigate soil sorption and subsequent desorption by dilution of methiozolin, as well as soil mobility using batch equilibrium experiments and thin-layer chromatography in nine root-zones. Evaluations focused on sand-based systems because they are used for construction of many golf course putting greens. Sorption coefficients ( $K_d$  values) ranged from 0.4 to 29.4 mL g<sup>-1</sup> and averaged 13.8 mL g<sup>-1</sup>. Sorption was most influenced by organic matter content; conversely, soil pH had a negligible effect. Methiozolin desorption did not occur with 0.01 M CaCl<sub>2</sub> dilution. Methiozolin mobility was low; retardation factors ( $R_f$  values) were < 0.05 for all media with ≥ 0.3 % organic matter. Sand (0.1% organic matter) resulted in an  $R_f$  value of 0.46. Overall, results indicated that ~24% of applied methiozolin was available for root uptake, and mobility was limited suggesting resistance to loss through leaching displacement.

Golf course managers frequently tank-mix fertilizers with herbicides to reduce time and labor, but no information is available regarding such mixtures with methiozolin. Research was conducted to evaluate methiozolin for annual bluegrass control and creeping bentgrass safety when tank-mixed with ammonium sulfate and iron sulfate. Mixtures with ammonium sulfate did not influence annual bluegrass control while reducing creeping bentgrass injury in some instances. Mixtures with iron sulfate varied by experimental run but annual bluegrass control was either similar or increased while creeping bentgrass injury did not vary by experimental run and was not influenced. Paclobutrazol resulted in similar control and injury with and without iron sulfate, and injury and control were similar to methiozolin at appropriate rates. In a comparison study of methiozolin, amicarbazone, and ethofumesate applied alone, with ammonium sulfate, and with iron sulfate, respectively, treatments including methiozolin and ethofumesate generally resulted in greater annual bluegrass control than those with amicarbazone. However, ethofumesate resulted in unacceptable turfgrass quality and NDVI reductions. Therefore

treatments including methiozolin were the best overall. While some differences were observed, generally annual bluegrass and creeping bentgrass response to these agrochemicals was not affected by tank-mix partner relative to that agrochemical applied alone.

## Acknowledgments

This work could not have been done without the help and support of many individuals. I am indebted and sincerely grateful to everyone who has helped. Specifically, I am tremendously thankful to Scott McElroy. I am greatly obliged to him for the impact he has had on my life and future career, and for his guidance. I am also very lucky to have an excellent graduate committee. Their expertise, guidance, and wisdom have been fortuitous. I hold them in high regard.

This work could also not have been possible without the love and support of my wife. I am especially thankful for everything she does to make my life better.

## Table of Contents

Abstract .....	ii
Acknowledgments.....	v
List of Tables .....	viii
List of Figures.....	x
List of Abbreviations .....	xi
Literature Review.....	1
Annual bluegrass ( <i>Poa annua</i> L.).....	1
Control options for annual bluegrass in turfgrass .....	3
General herbicide bioavailability in soils .....	9
Methiozolin.....	12
Dissertation objectives .....	15
Methiozolin Absorption and Translocation in Annual Bluegrass.....	17
Introduction.....	17
Materials and Methods.....	20
Results and Discussion .....	25
Methiozolin Sorption and Mobility in Sand-Based Root-Zones .....	40
Introduction.....	40
Materials and Methods.....	43
Results and Discussion .....	49
Annual Bluegrass ( <i>Poa annua</i> ) Control with Methiozolin Tank-Mixed with Nutrients .....	63

Introduction.....	63
Materials and Methods.....	65
Results and Discussion .....	68
Broad Implications for Methiozolin Field Application .....	90
Literature Cited .....	96

## List of Tables

Table 1. Preemergence annual bluegrass control from methiozolin and indaziflam in two combined greenhouse experiments in Auburn, AL in 2011 to 2012 .....	33
Table 2. Postemergence annual bluegrass control 25 days after treatment in two combined greenhouse experiments in Auburn, AL in 2011 to 2012.....	34
Table 3. Postemergence annual bluegrass control from methiozolin as influenced by rate, growth stage, and treatment placement in two combined greenhouse experiments in Auburn, AL in 2011 to 2012 .....	35
Table 4. Postemergence annual bluegrass control from indaziflam as influenced by growth stage and treatment placement in two combined greenhouse experiments in Auburn, AL in 2011 to 2012.....	36
Table 5. Foliar absorption and translocation of <sup>14</sup> C-methiozolin by annual bluegrass over time from two combined laboratory experiments .....	37
Table 6. Root absorption and translocation of <sup>14</sup> C-methiozolin by annual bluegrass over time in two combined laboratory experiments.....	38
Table 7. Chemical and physical properties of soils used for methiozolin absorption, desorption, and mobility studies .....	55
Table 8. Soil sorption coefficient ( $K_d$ ), organic carbon coefficient ( $K_{oc}$ ), and percent methiozolin in solution values for batch equilibrium sorption experiments with methiozolin at 3 mg kg soil <sup>-1</sup> .....	56
Table 9. Soil sorption ( $K_d$ ) and soil organic carbon sorption ( $K_{oc}$ ) coefficients of herbicides related to soil activity.....	57
Table 10. Effect of pH on soil sorption coefficients ( $K_d$ ) for field soil and USGA sand based root-zone with methiozolin added at 3 mg kg soil <sup>-1</sup> .....	58
Table 11. Comparison of methiozolin soil sorption (%) after initial sorption and subsequent dilution desorption steps in batch equilibrium experiments with 0.1 M CaCl <sub>2</sub> .....	59
Table 12. Methiozolin and isoxaben retardation factors ( $R_f$ ) determined by thin-layer soil chromatography .....	60



Table 13. Annual bluegrass control rated at various weeks after initial treatment (WAIT) from herbicide and iron sulfate tank-mixes from two field experiments conducted in Auburn, AL in 2010-11 and 2014. .... 81

Table 14. Creeping bentgrass injury rated at various weeks after initial treatment (WAIT) from herbicide and iron sulfate tank-mixes from two field experiments conducted in Auburn, AL in 2010-11 and 2014. .... 82

Table 15. Annual bluegrass control rated at various weeks after treatment from herbicide and fertilizer tank-mixes from two field experiments conducted in Auburn, AL in 2011-12 and 2014 ..... 83

Table 16. Turfgrass quality rated at various weeks after treatment from herbicide and fertilizer tank-mixes from two field experiments conducted in Auburn, AL in 2011-12 and 2014..... 84

Table 17. Turfgrass normalized difference vegetative index (NDVI) rated at various weeks after treatment from herbicide and fertilizer tank-mixes from two field experiments conducted in Auburn, AL in 2011-12 and 2014..... 85

Table 18. Pearson's correlation coefficients between turfgrass quality and normalized difference vegetative index (NDVI) from two field experiments conducted in Auburn, AL in 2011-12 and 2014..... 86

## List of Figures

Figure 1. Methiozolin chemical structure .....	15
Figure 2. Annual bluegrass after treatment with <sup>14</sup> C-methiozolin.....	39
Figure 3. Methiozolin soil sorption from batch equilibrium experiments as a function of time for new USGA sand and field soil with nonlinear regression analysis .....	61
Figure 4. Relationship between methiozolin soil sorption (%) and organic matter content (%) for USGA sand with 0, 2.5, 5.0, 10, or 20% (w/w) peat in batch equilibrium sorption experiments .. .....	62
Figure 5. Annual bluegrass control from methiozolin applied three times sequentially at 0.84, 1.68, and 3.36 kg ai ha <sup>-1</sup> from two combined field experiments in Auburn, AL in 2012 and 2014 .. .....	87
Figure 6. Creeping bentgrass injury from methiozolin applied three times sequentially at 0.84, 1.68, and 3.36 kg ai ha <sup>-1</sup> with and without ammonium sulfate at 12.2 kg N ha <sup>-1</sup> from two combined field experiments in Auburn, AL in 2012 and 2014. ....	88
Figure 7. Annual bluegrass control from methiozolin applied three times sequentially at 0.84, 1.68, and 3.36 kg ai ha <sup>-1</sup> from two combined field experiments in Auburn, AL in 2010-11 and 2014.....	89
Figure 8. Profiles of United States Golf Association (USGA) specified root-zones for putting greens .....	94

## List of Abbreviations

ai	active ingredient
AL	Alabama
Bq	Becquerel
C	centigrade
CaCl <sub>2</sub>	calcium chloride
CI95	95% confidence interval
cm	centimeter
d	day
DAT	days after treatment
dpm	disintegration per minute
FeSO <sub>4</sub>	iron sulfate
g	gram
ha	hectare
HAT	hours after treatment
H <sub>2</sub> O	water
K	potassium
K <sub>d</sub>	Soil sorption coefficient
K <sub>oc</sub>	organic carbon coefficient
K <sub>ow</sub>	octanol:water partitioning coefficient
L	liter

μg	microgram
mL	milliliter
μL	microliter
mm	millimeter
N	nitrogen
NC	North Carolina
NY	New York
OH	Ohio
P	phosphorous
PA	Pennsylvania
POST	postemergence
ppm	part per million
PRE	preemergence
R <sub>f</sub>	retardation factor
SAS	Statistical Analysis Software
TAT	tyrosine aminotransferase
USGA	United States Golf Association
vol vol <sup>-1</sup>	volume per volume
wk	week
wt wt <sup>-1</sup>	weight per weight

## Literature Review

### Annual Bluegrass (*Poa annua* L.)

**Description.** Annual bluegrass (*Poa annua* L.) is a member of the Poaceae and a native of Europe (Warwick 1979). The grass subsists as an annual or perennial (Beard et al. 1978; Callahan and McDonald 1992; Gibeault and Goetze 1972; McElroy et al. 2004b). Annual bluegrass is tufted. Its stems can have two to four nodes when mature that may root at the nodes. Leaves and stems are mostly glabrous and light, bright to dark green in color. Ligules are present and thinly membranous. The leaf blade forms a smooth v-shape with a boat-shaped tip, characteristic of its genus. Annual bluegrass' inflorescence occurs on a terminal panicle that is light green. The fruit is a tan, light brown color upon maturity. The roots are fibrous. Annual bluegrass can form dense mats (Beard 1973; Beard et al. 1978; Mitich 1998; Warwick 1979).

Annual bluegrass is an allotetraploid ( $2n = 4x = 28$ ) with presumed progenitor species of *Poa supina* Schrad. ( $2n = 2x = 14$ ) and *Poa infirma* Kunth. ( $2n = 2x = 14$ ) (Tutin 1957; La Mantia and Huff 2011). Annual bluegrass is known to have worldwide distribution, with extensive ecotypic variation (Lush 1989; McElroy et al. 2002; Tutin 1957). The adaptability of annual bluegrass is most easily illustrated by the fact that it is one of the few species to colonize Antarctica and is also a major weed species in the Southeastern United States (Greene and Walton 1975; Olech 1996; Walton and Smith 1973; Webster 2004).

**Weediness.** Annual bluegrass possesses many traits that make a problematic weed. It is a highly adaptable species that exhibits a wide range of phenotypic and genotypic variability (Lush 1989; Mitich 1998). Annual bluegrass is tolerant of many soil types, from clays to sands. However, it is

not tolerant of strongly acidic soils (Beard 1970; Gibeault 1966). Annual bluegrass generally has a short life-cycle and produces large amounts of seed; estimates range from 1,050 to 2,250 seeds per plant (Holm et al. 1997). Even plants mown at 0.5 cm are capable of producing 360 seeds (Beard 1973). Seeds remain viable for 6 years or longer, ensuring a large soil seed-bank (Roberts and Feast 1973). The grass is completely self-compatible (Warwick 1979). Seeds germinate and establish across a wide range of environmental conditions (Wells 1974). Seedlings grow rapidly during the fall and winter of warmer climates (Younger 1959). Annual bluegrass occurs in pastures, agronomic crops, horticulture crops, home lawns, as well as golf courses (Mitich 1998). The weed is also tolerant of high traffic, making it a common weed in these areas (Mitich 1998). Annual bluegrass is not tolerant of air pollution, drought, and temperature extremes (Beard 1970; Gibeault 1966).

Annual bluegrass is a problematic cool-season weed in turfgrass throughout the southeastern United States (Gibeault 1966). Annual bluegrass disrupts both the aesthetics and playability of turfgrass via its clump-like growth habit, ability to form panicles at low mowing heights, prolific seed-head production, and light green color (Beard 1973; Mitich 1998). Two biotypes of annual bluegrass exist: the annual type (*Poa annua* var. *annua* L. Timm.) and the perennial type [*Poa annua* var. *reptans* (Hauskins) Timm.] (Mitich 1998; Tutin 1957). Annual bluegrass infests both warm- and cool-season turfgrasses where it competes for water and nutrients. It can be especially unsightly in dormant warm-season grasses due to its green color amongst the brown, dormant turfgrass. Additionally, warm-season turfgrass dormancy coincides with active annual bluegrass growth, giving a competitive advantage to the weed (Bingham et al. 1969; Johnson 1980).

## **Control options for annual bluegrass in turfgrass**

Annual bluegrass control is difficult to achieve due to its extensive weediness traits.

Annual bluegrass control is best achieved through a combined agronomic approach of proper cultural and chemical practices (McCarty 2005). Best management practices are outlined below.

**Cultural.** Annual bluegrass populations can be reduced with proper cultural practices. Cultural practices are anything done to the turfgrass as a part of regular maintenance. Cultural practices that inhibit annual bluegrass proliferation include proper fertility, soil pH, soil moisture, mowing height, and competition with other species. Cultural weed control practices are becoming increasingly important with pesticide bans, such as those in Canada (Elford et al. 2008).

Proper fertility is one cultural practice that can be used to discourage annual bluegrass populations. N, P, and K are the largest contributors to annual bluegrass growth, in that order (Juska and Hanson 1969). N application has resulted in increased annual bluegrass populations, and regular N application has resulted in more robust populations (Beard et al. 1978; Dest and Allinson 1981). K and P fertilization favors annual bluegrass infestation, and their effects have been found to mutually benefit the other (Waddington et al. 1978). Routine P application has resulted in maximized stand density and survival of annual bluegrass (Dest and Allinson 1981). Micronutrients including calcium, sulfur, and zinc may also play significant roles in annual bluegrass competitiveness and population health (Bristow et al. 2011; Kuo 1993; Varco and Sartain 1966; Xu and Mancino 2001). Generally, low P fertilization and avoiding N application during annual bluegrass germination are the best practices for discouraging the weed (LeStange et al. 2012; McCarty 2005; Varco and Sartain 1966).

Soil pH influences the availability of many nutrients and can be managed to reduce annual bluegrass populations (Juska and Hanson 1969; Kuo 1993; Varco and Sartain 1966).

Varco and Sartain (1966) found annual bluegrass germination and growth to be inhibited by pH < 5.3. Overall, annual bluegrass populations are best managed (reduced) with limited P applications and soil pH approximately 5.0, provided the desirable turfgrass is tolerant to these practices (Varco and Sartain 1966).

Annual bluegrass is competitive at many mowing heights and is tolerant of extremely low mowing heights, typical of golf course putting greens (Beard 1973). Despite this tolerance, reducing mowing height has decreased annual bluegrass cover in creeping bentgrass fairways (Pierce et al. 1987). Clipping removal (as opposed to returning clippings to the turfgrass) has resulted in decreased annual bluegrass populations and a decreased seedbank (Beard et al. 1978; Gaussoin and Branham 1989). In a factorial study of clipping fate, growth regulator application, and overseeding, Gaussoin and Branham (1989) found clipping removal, overseeding, and no growth regulator application to be the best treatment to reduce the annual bluegrass population and increase creeping bentgrass.

Soil moisture levels can also be managed to discourage annual bluegrass germination and encroachment. Annual bluegrass is associated with high soil moisture levels as well as short and frequent irrigation cycles. Long, infrequent irrigation best favors more deep-rooted desirable turfgrass over the more shallow-rooted, opportunistic annual bluegrass (LeStrange et al. 2012; McCarty 2005).

Turfgrass managers often overseed with perennial ryegrass (*Lolium perenne* L.) and other species during warm-season turfgrass dormancy to increase aesthetics, wear tolerance, utility, and achieve green winter color (McCarty 2005). Bingham et al. (1969) found that annual ryegrass (*Lolium multiflorum* Lam.) reduced annual bluegrass by 80 to 90% more than other species used for overseeding. Overseeding reduces annual bluegrass infestations via competition,



but does not completely control the weed (Elford et al. 2008). Therefore, herbicidal control options are necessary to maintain a sward free of annual bluegrass (Baldwin 1993; Bingham et al. 1969).

**Chemical.** Many chemical control options are available to turfgrass managers for annual bluegrass control. These include fumigants, growth regulators, PRE and POST herbicides, and nonselective herbicide options.

*Fumigants.* When turfgrass areas are being established or renovated, soil fumigants are often used to ensure a weed-free area. Many soil fumigants may have the added benefits of reducing the soil seedbank, pathogens, and other weeds species beyond annual bluegrass. Dazomet is one example of a fumigant used to reduce or eliminate annual bluegrass populations (Park and Landschoot 2003).

*Growth regulators.* Annual bluegrass control is widely managed through a combination of proper cultural practices and the use of plant growth regulators (Gaussoin and Branham 1989; LeStrange et al. 2012; McCarty 2005). These management strategies can successfully shift the competitive advantage from the weed to the desirable turfgrass, but many times they do not result in complete annual bluegrass control. Additionally, plant growth regulators require frequent and multiple applications that increase time and application costs (Dickens 1979; Johnson and Murphy 1996; Johnson and Murphy 1996; Woosley et al. 2003). Therefore, other control options, such as herbicidal control, are necessary to maintain turfgrass completely free of annual bluegrass.

Suppression, but not control, has been reported with the plant growth regulators paclobutrazol and flurprimidol (Johnson and Murphy 1995; Johnson and Murphy 1996; Woosley

et al. 2003). Paclobutrazol and flurprimidol are the only options available for use in putting greens.

*Preemergence herbicides.* Annual bluegrass control can be achieved with the use of preemergence (PRE) herbicides, which control germinating annual bluegrass. However, the long germination period can outlast the activity of some PRE herbicides, requiring multiple applications to achieve complete, season-long control (Beard et al. 1978; Itoh et al. 1997; Kaminski and Dernoeden 2007; McElroy et al. 2004b). Furthermore, annual bluegrass can exist as a perennial, making PRE control impractical (Callahan and McDonald 1992; Gibeault and Goetze 1972; McElroy et al. 2004b).

PRE annual bluegrass control has been successful with a variety of herbicides including dinitroaniline herbicides (Bingham and Shaver 1979) such as prodiamine and pendimethalin, cellulose biosynthesis inhibiting herbicides including indaziflam (Brosnan et al. 2012a; Myers et al. 2009; Perry et al. 2011a), protophyrinogen IX oxidase inhibiting herbicides including flumioxazin (Flessner et al. 2011) and oxadiazon (Bingham and Shaver 1979), as well as a number of antiquated chemistries (Bingham et al. 1969; Dickens 1979; Goss 1964).

Herbicidal annual bluegrass control options are limited in overseeded situations. PRE herbicides are difficult to utilize without overseeded turfgrass injury or reduced germination due to the similar germination periods of overseeded turfgrasses and annual bluegrass (Bingham et al. 1969; Johnson 1983). Therefore, many times PRE control is impractical in overseeded situations.

Herbicides registered in the United States of America for PRE annual bluegrass control in putting greens are limited. Often, a herbicide label does not explicitly say whether the product can be used in putting greens. Currently, only bensulide and fenarimol are explicitly allowed for

PRE annual bluegrass control in creeping bentgrass putting greens (Anonymous 2009a; Anonymous 2010). Additionally, rimsulfuron is registered for use in bermudagrass putting greens only (Anonymous 2009b).

*Postemergence herbicides.* Selective annual bluegrass control can also be achieved with postemergence (POST) herbicides. POST control can also be complicated by the long germination period of annual bluegrass that may exceed the effectiveness period of POST herbicides. Therefore, multiple applications of POST herbicides may be necessary for complete control (Beard et al. 1978; Itoh et al. 1997; McElroy et al. 2004b). Additionally, POST herbicides are also difficult to utilize when turfgrass is overseeded due to similar herbicide tolerance of commonly used species for overseeding and annual bluegrass (Coats and Krans 1986; McElroy et al. 2011; Yelverton and McCarty 2001).

Sulfonylurea herbicides such as foramsulfuron, trifloxysulfuron, rimsulfuron, and others are POST herbicides that are commonly used to control annual bluegrass and other weeds in warm-season turfgrasses (Toler et al. 2007; Wehtje and Walker 2002). The pyrimidinyl benzoate herbicide bispyribac-sodium (bispyribac), and the imidazolinone herbicide, imazaquin, can be used to control annual bluegrass; however, multiple applications are needed for acceptable control (Lycan and Hart 2006; McElroy et al. 2011; Rodriguez et al. 2001). Photosystem II inhibiting herbicides such as simazine, atrazine, and amicarbazone have also resulted in annual bluegrass control (Perry et al. 2011b).

The plant growth regulator ethofumesate is commonly applied for annual bluegrass control when bermudagrass is overseeded with perennial ryegrass. Ethofumesate can be effective, but complete control is difficult to achieve (Coats and Krans 1986; Dickens 1979; Johnson 1983; Woosley et al. 2003).

Non-traditional herbicidal control options also exist. One example is the use of non-selective herbicides such as glyphosate, glufosinate, paraquat, and others when warm-season turfgrasses are fully dormant (Toler et al. 2007; Binkholder et al. 2011).

Recently, glyphosate-tolerant perennial ryegrass cultivars have been conventionally bred, potentially allowing glyphosate to be used as a selective weed control agent within these cultivars (Aldahir et al. 2011a, b; Flessner et al. 2014). Genetically modified turfgrasses exist for glyphosate tolerance; however, it is extremely unlikely these cultivars will be commercially released due to regulatory and public concerns (Gardner et al. 2004).

Similar to PRE annual bluegrass control in putting greens, POST herbicide options are limited. Again, herbicide product labeling is sometimes vague. Currently, rimsulfuron is the only herbicide with direct labeling for annual bluegrass control in bermudagrass putting greens in the United States (Anonymous 2009b).

*Resistance to herbicides.* Annual bluegrass herbicide resistance has developed for many modes-of-action including PRE and POST herbicides. This fact further complicates control options for many turfgrass managers. Resistance has been reported to photosystem II inhibitors (amicarbazone, atrazine, simazine, diuron), photosystem I inhibitors (paraquat), inhibitors of very long chain fatty acids (ethofumesate), mitotic inhibiting herbicides (prodiamine, pendimethalin), acetolactate synthase inhibitors (foramsulfuron, trifloxysulfuron, bispyribac, imazaquin) and 5-enolpyruvate shikimate-3-phosphate inhibitors (glyphosate) (Anonymous 2012a; Binkholder et al. 2011; Brosnan, et al. 2012b; Cutulle et al. 2009; Hanson and Mallory-Smith 2000; Isgrigg et al. 2002; Kelly et al. 1999; Heap 1997; Hutto et al. 2004; McElroy et al. 2013; Perry et al. 2012).

*Biological.* Bioherbicides for annual bluegrass control have been evaluated, but currently there are no commercial options. Johnson (1994) reported 82 and 64% annual bluegrass control in late April following three sequential applications in February and March with two *Xanthomonas campestris* pv. *Poannua* isolates.

*Fungicides.* The fungicide fenarimol can be used to reduce or control annual bluegrass populations (Anonymous 2010). McElroy et al. (2004b) reported fenarimol did not affect germination of annual bluegrass, but did decrease root length. Fungal pathogen prevention and control are added benefits to using fungicides with annual bluegrass activity (Anonymous 2010).

### **General herbicide bioavailability in soils**

Herbicide-soil interactions are governed by many forces, and the interaction is largely dependent on the herbicide and soil in question. A complete review of all herbicide-soil interactions is beyond the scope of this dissertation. An excellent review for pesticide binding to soils is Gevao et al. (2000).

Some generalities can be made in regards to pesticide-soil interactions. Generally, as organic matter increases, herbicide adsorption increases, resulting in less herbicide efficacy (Harris 1966; Stougaard et al. 1990). Upchurch and Mason (1962) reported a strong, positive correlation between cation exchange capacity, exchangeable calcium, organic matter, moisture equivalent free drainage value, and total exchangeable bases and phytotoxicity, regardless of herbicide. Overall, the researchers came to the conclusion that the soil organic matter was the most important factor influencing herbicide phytotoxicity (Upchurch and Mason 1962). Jacques and Harvey (1979), while studying several dinitroaniline herbicides, also found soil organic matter to be most closely associated with herbicide adsorption. Harrison et al. (1976) measured

phytotoxicity of five herbicides and reported that organic matter was most highly correlated of fifteen soil properties evaluated. Indeed, many studies have reached this conclusion (Harris and Sheets 1965; Nearpass 1965; Obrigawitch et al. 1981; Talbert and Fletchall 1965; Weber et al. 1969). Maximum adsorbance of ionizable herbicides is at or near its pKa (Stougaard et al. 1990; Weber et al. 1969). It should be noted that no soil property is predictive of all soils and herbicide efficacies (Harris and Sheets 1965; Nearpass 1965).

Soil texture also results in a general trend in herbicide-soil interactions. Clay particles are negatively charged and attract cations (Tisdale et al. 1985). As clay content increases, adsorption of positively charged (cationic) herbicide molecules generally increases (Goetz et al. 1989; Patterson et al. 1982). Increased adsorption to soil colloids generally decreases herbicide mobility and efficacy (Stougaard et al. 1990). Talbert and Fletchall (1965) reported increased adsorption with increased clay content of the *s*-triazine herbicides. When the herbicide molecule acts as an anion, increasing clay content has resulted in less soil-herbicide sorption. Wehtje et al. (1987) reported decreasing sorption of sulfometuron and imazapyr as clay content increased.

Soil pH also plays an important role in pesticide-soil interactions. Generally, pH and herbicide adsorption are inversely related for anionic or basic herbicide molecules or herbicides that are readily protonated (Stougaard et al. 1990). This relationship exists because when a herbicide molecule is protonated, it becomes more attracted to negatively charged soil particles. The result is that with lower pH, protonation is more likely to occur, leading to greater attraction to negatively charged soil particles, which increases herbicide adsorption. This relationship has been reported for atrazine, simazine, imazaquin, imazethapyr, chlorimuron, metribuzin, sulfometuron, and imazapyr (Best and Weber 1974; Best et al. 1975; Goetz et al. 1986, 1989; Kells et al. 1980; Ladlie et al. 1976a, b; McGlamery and Slife 1966; Renner et al. 1988; Talbert

and Fletchall 1965; Wehtje et al. 1987). The result of greater adsorption to soil particles at lower pHs is less bioavailability, which results in decrease herbicide efficacy (Best and Weber 1974; Best et al. 1975; Ladlie et al. 1976a, b; Stougaard et al. 1990). The level of pH response typically varies according to herbicide (Best and Weber 1974; Harris 1967; Renner et al. 1988; Stougaard et al. 1990; Wehtje et al. 1987). In some cases, herbicide efficacy is not influenced by soil pH or influenced to a very small degree (Farmer and Aochi 1974; Nearpass 1965; Renner et al. 1988). Harris (1967) also noted that adsorption of some herbicides is largely influenced by soil pH while others are not. The triazine herbicides are well known for their relationship with soil pH. Triazine persistence in soil increases with decreasing soil pH (Best and Weber 1974; Hiltbold and Buchanan 1977; Kells et al. 1980; Ladlie et al. 1976a). Adsorption of ionizable herbicides is greatest near its pKa (Ladlie et al. 1976a, b; Stougaard et al. 1990).

Soil moisture can influence herbicide availability for root uptake and therefore affect root absorbed herbicide efficacy. Generally in soils that retain moisture, more herbicide is in soil solution and available for root uptake; however, herbicide concentration in soil solution and water uptake rate by the plant are both low (Osgerby 1974).

Adsorption to soil particles also influences the soil mobility of the herbicide. As a herbicide becomes more adsorbed, it generally becomes less mobile in the soil (Best and Weber 1974; Ladlie et al. 1976a, b; Stougaard et al. 1990). This effect results in less leaching from the soil, and also explains why clay soils are less likely to leach herbicides into the ground water than sandy soils.

Herbicide water solubility and octanol-water partitioning coefficient ( $K_{ow}$ ) are predictive of soil sorption and mobility. Methiozolin has a water solubility of 3.4 mg L<sup>-1</sup> and a log $K_{ow}$  value of 3.9, indicating that it is hydrophobic (Koo et al. 2010; Anonymous 2012b). Methiozolin does

not have an ionizable functional group. Uncharged herbicides with high water solubility and low  $K_{ow}$  tend to be poorly sorbed to soil and are therefore more mobile due to their greater affinity for the water phase. Hydrophobic herbicides with high  $K_{ow}$  values such as methiozolin tend to sorb to soil rather than stay in soil solution and are therefore less mobile (Ross and Lembi 1999; Sakaliene et al. 2007). However, herbicides that are charged may not adhere to these trends. Glyphosate, which typically exists as a cation or zwitterion, has high water solubility but sorbs to soils due to electrostatic attraction (Al-Rajab et al. 2008; Sprankle et al. 1975).

### **Methiozolin.**

Methiozolin is potentially a ground breaking herbicide for annual bluegrass control in putting greens due to its PRE and selective POST annual bluegrass activity and safety to creeping bentgrass (*Agrostis stolonifera* L.) and ultradwarf bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burt Davy] putting greens. Methiozolin is the first herbicide to be commercialized of the isoxazoline chemistry class. The herbicide has been reported to selectively control several annual grass weed species including barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], goosegrass [*Eleusine indica* (L.) Gaertn.], and annual bluegrass (Brosnan et al. 2013a; Han and Kaminski 2011; Hwang and Koo 2009; Hwang et al. 2005; Koo et al. 2010; Koo et al. 2014; McNulty and Askew 2011; Nam et al. 2012; Norsworthy et al. 2011). Methiozolin reportedly controls annual bluegrass PRE and selective POST through multiple, timely applications (Brosnan et al. 2013a; Han and Kaminski 2011; Hwang and Koo 2009; McNulty and Askew 2011; Nam et al. 2012). Safety of methiozolin to creeping bentgrass may be attributed to reduced absorption relative to annual bluegrass; McCullough et al. (2013) also reported methiozolin is both root and foliar absorbed in both grasses. Notably, no metabolism of



the parent compound was observed in either grass, indicating that differential metabolism is not responsible for the selectivity of methiozolin (McCullough et al. 2013).

Methiozolin is reported to inhibit cell wall biosynthesis which was evaluated using maize (*Zea mays* L.) (Lee et al. 2007). However, the exact site/enzyme of inhibition is believed to be different from that of other cell wall biosynthesis inhibiting herbicides (Hwang et al. 2005; McNulty and Askew 2011; Nam et al. 2012). Methiozolin has also been reported to inhibit tyrosine aminotransferase (TAT); this inhibition blocks the production of plastoquinone and tocopherol, both of which are important in the protection of membranes to oxidative stresses (Grossmann et al. 2012). TAT inhibition research was conducted using nongraminaceous (i.e., *Lemna paucicostata* L. and *Arabidopsis thaliana* L.), and thus may have a different mode-of-action in grasses (Grossmann et al. 2012). These modes-of-action may not be mutually exclusive.

Methiozolin has a chiral center in its molecular structure. Similar to the herbicide metholachlor, only one optical isomer has herbicidal activity (Böger et al. 2000; Senseman 2007). Nam et al. (2012) reported that the *S*-isomer of methiozolin resulted in excellent PRE annual bluegrass control while the *R*-isomer had no noticeable herbicidal activity.

Methiozolin has a water solubility of 3.4 mg L<sup>-1</sup> and a logK<sub>ow</sub> value of 3.9, indicating that it is hydrophobic (Koo et al. 2010; Anonymous 2012b). Methiozolin does not have an ionizable functional group (Figure 1).

PRE annual bluegrass control requires longevity of methiozolin in the field for adequate, season-long control, which may be an issue. In laboratory studies, Hwang et al. (2013) reported the soil half-life of methiozolin to be approximately 49 days under aerobic conditions, but a half-life could not be estimated under anaerobic, flooded conditions due to limited degradation. In field studies, Norsworthy et al. (2011) reported that methiozolin PRE activity on barnyardgrass

ceased six weeks after application. PRE control is also dependent on herbicide availability in the soil; soil herbicide availability is known to depend on soil texture, organic matter, and pH, predominately (Goetz et al. 1986; Goetz et al. 1989; Ogle and Warren 1954; Patterson et al. 1982; Upchurch and Mason 1962). Hwang and Koo (2009) previously found PRE annual bluegrass control from methiozolin to be independent of soil texture (loam sand, sandy loam, or clay loam). McCullough and Gómez de Barreda (2012) reported that reseeding creeping bentgrass, perennial ryegrass (*Lolium perenne*) and tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.] should be delayed approximately 4, 4 and 2 weeks after methiozolin application at 1.12 kg ai ha<sup>-1</sup>, respectively, which may also reflect PRE activity of methiozolin.

POST annual bluegrass control with methiozolin is reported to require multiple applications (Brosnan et al. 2013a; Han and Kaminski 2011; McCullough et al. 2013). In a POST selective placement study, Brosnan et al. (2012a) reported that annual bluegrass control was best with soil-only and foliar-plus-soil applications rather than foliar-only applications, which is indicative of root absorption (also noted above by McCullough et al. 2013). Koo et al. (2014) reported that no herbicidal activity resulted from leaf-only application. Additionally, annual bluegrass maturity is a factor in control, with larger, more mature stages being more difficult to control. Hwang and Koo (2009) reported 80 and 60% growth inhibition of two- and four-leaf stage annual bluegrass from methiozolin applied at 500 g ha<sup>-1</sup>, respectively. Temperature has been reported to influence methiozolin safety to creeping bentgrass. McCullough et al. (2012) reported that creeping bentgrass injury was observed in field studies from February/March applications and in growth chamber experiments where injury was two- and four-fold greater at 20 and 30 C, respectively, compared to 10 C. This increase injury may be explained by a two-

fold increase in translocation of methiozolin observed in laboratory experiments as 30/25 C compared to 15/10 C (day/night) (McCullough et al. 2012). Methiozolin absorption in annual bluegrass was influenced by temperature; 15% of applied methiozolin was absorbed 72 hours after treatment at 15/10 C (day/night) compared to 35% at 30/25 C (McCullough et al. 2013).

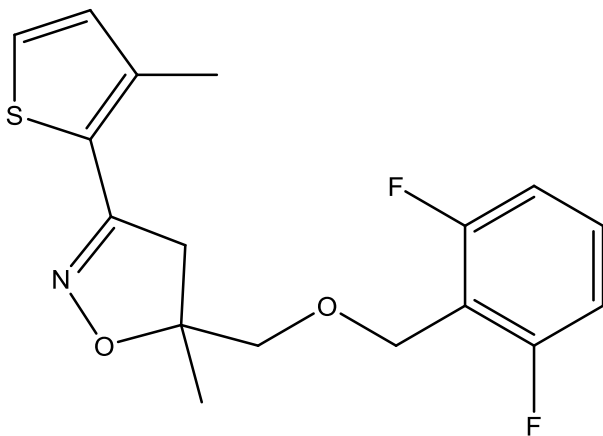


Figure 1. Methiozolin chemical structure.

### **Dissertation objectives.**

Research was conducted to investigate the sorptive capacity of methiozolin to soil constituents, and the effect of the sorptive nature on bioavailability across four objectives.

1) Absorption and translocation of foliar and root applied methiozolin by annual bluegrass. Research was conducted to evaluate absorption and translocation of methiozolin when applied to both foliage and roots. Additional selective placement studies were conducted to assess annual bluegrass control from foliar-only, soil-only, and foliar-plus-soil application placement.

2) Retention and release of methiozolin in soil solution as influenced by organic matter.

Research was conducted to investigate impact of organic matter content of constructed sand based root-zones on methiozolin retention. Further research in this objective was to collect soils from constructed sand-based root-zones and evaluate them for comparative methiozolin sorption.

3) Effect of soil pH on methiozolin retention and bioavailability. Herbicide sorptive capacity and bioavailability are often influenced by soil pH level. Golf course putting greens can be managed at different pH levels, which often vary due to sand source utilized for root-zone construction and acidifying capabilities of fertilizers utilized. Further, many golf courses purposely decrease soil pH to discourage *Poa annua* growth. Two studies were conducted to evaluate this research. First, sorptive capacity/retention studies were conducted (similar to objective 2) to investigate the retention of methiozolin at pH levels of 5.0, 6.0, 7.0, and 8.0 to two different soils. A second study a soil versus foliar control study was conducted to investigate *Poa annua* control when methiozolin is soil applied at these pH levels.

4) Downward movement of methiozolin as influenced by organic matter and soil type.

Downward movement of methiozolin through the soil profile could like decrease efficacy due to leaching from the soil profile. Research was conducted to evaluate seven soils on their influence on downward movement of methiozolin through the soil profile.

5) Field studies were conducted to assess compatibility of methiozolin with common tank-mix partners such as fertilizers for annual bluegrass control and creeping bentgrass safety.

## **Methiozolin Absorption and Translocation in Annual Bluegrass (*Poa annua*)**

[This chapter has been updated from its published form, Flessner, M.L., G.R. Wehtje, and J.S. McElroy. 2013. Methiozolin absorption and translocation in annual bluegrass (*Poa annua*). Weed Sci. 61:201–208, to include content involving indaziflam.]

### **Introduction**

Annual bluegrass (*Poa annua* L.) is a problematic turfgrass weed due to its prolific seedhead production, bunch-type growth habit, color, and other characteristics (Beard 1973). Annual bluegrass is especially troublesome in golf course putting greens due to its ability to survive and thrive at very low mowing heights. These factors disrupt turfgrass utility and aesthetics.

Annual bluegrass control in putting greens is currently managed through a combination of proper cultural practices and the use of plant growth regulators, such as paclobutrazol and flurprimidol (Dickens 1979; Gaussoin and Branham 1989; Johnson and Murphy 1996; Johnson and Murphy 1996). These management strategies can successfully shift the competitive advantage away from annual bluegrass to the desirable turfgrass. However, these strategies do not result in complete annual bluegrass control. Additionally, plant growth regulators require frequent and multiple applications. Therefore, herbicidal control options are necessary to maintain putting greens free of annual bluegrass.

Selective herbicidal annual bluegrass control options are limited in putting greens and complicated by several factors. Annual bluegrass persists as various ecotypes and biotypes including a perennial, making herbicidal preemergence (PRE) control problematic (Callahan and McDonald 1992; Gibeault and Goetze 1972; McElroy et al. 2004b). Additionally, herbicide-resistant strains exist to many modes-of-action, which may interfere with PRE and postemergence (POST) control (Anonymous 2012a; Heap 1997; McElroy 2012). Currently in putting greens in the United States, only bensulide and fenarimol are explicitly labeled for PRE control; only rimsulfuron is explicitly labeled for POST control in bermudagrass greens (Anonymous 2009a; Anonymous 2009b; Anonymous 2010).

Methiozolin is a herbicide of the isoxazoline chemistry class that has been reported to selectively control several annual grass weed species including barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], goosegrass [*Eleusine indica* (L.) Gaertn.], and annual bluegrass (Brosnan et al. 2012a; Han and Kaminski 2011; Hwang and Koo 2009; Hwang et al. 2005; McCullough and Gómez de Barreda 2012; McNulty and Askew 2011; Nam et al. 2012; Norsworthy et al. 2011). Methiozolin reportedly controls annual bluegrass PRE and POST through multiple, timely applications (Haguewood et al. 2012; Han and Kaminski 2011, 2012; Hoyle et al. 2012; Hwang and Koo 2009; Koo et al. 2014; McNulty and Askew 2011; Nam et al. 2012; Trappe et al. 2012). Methiozolin has been reported to inhibit tyrosine aminotransferase (TAT); this inhibition blocks the production of plastoquinone and tocopherol, both of which are important in membrane protection to oxidative stresses (Grossmann et al. 2012). TAT inhibition research was conducted using nongraminaceous plants (Grossmann et al. 2012). Another proposed mode-of-action for methiozolin is cell wall biosynthesis inhibition, which was established using maize (*Zea mays* L.) (Lee et al. 2007). However, the exact site/enzyme of inhibition is believed to be different

from that of other cell wall biosynthesis inhibiting herbicides (Hwang et al. 2005; Lee et al. 2007; McNulty and Askew 2011; Nam et al. 2012).

Methiozolin has an octanol-water partitioning ( $\log K_{ow}$ ) value of 3.9, indicating that it is lipophilic (Hess and Foy 2000; S. J. Koo, personal communication). This value is useful for predicting absorption and to a lesser extent translocation properties of herbicides. Foliar absorption of lipophilic herbicides occurs readily via diffusion through the cuticle (Hess and Foy 2000). Root absorption of lipophilic herbicides occurs primarily through the symplastic pathway (Briggs et al. 1982; Hess and Foy 2000; Hsu et al. 1990). Greater lipophilicity also results in greater affinity for membranes (Hsu et al. 1990). Herbicides with  $\log K_{ow}$  values greater than 3.5, such as methiozolin, have excellent membrane permeability, but do not enter the xylem sap well due to low water solubility.

Among herbicides that can selectively control annual bluegrass in turfgrass, control varies with herbicide placement. Brosnan et al. (2012a) reported that soil-only and foliar-plus-soil methiozolin application reduced annual bluegrass biomass greater and resulted in greater control than foliar-only application. These authors concluded that methiozolin is a root absorbed herbicide (Brosnan et al. 2012a). Other researchers have noted annual bluegrass control varies with herbicide placement with other herbicides. Perry et al. (2011b) reported that annual bluegrass control with amicarbazone and atrazine was greater (> 95%) from soil-only applications than control from foliar-only applications (< 60%). Wehtje and Walker (2002) reported greater annual bluegrass control from soil-only and foliar-plus-soil applied rimsulfuron compared to foliar-only application. Optimal herbicide placement for annual bluegrass control likely varies simply according to herbicide.

POST annual bluegrass control with methiozolin generally requires sequential or multiple applications and visual symptoms are generally not evident until 10 to 20 d after application (Brosnan et al. 2012a; Haguewood et al. 2012; Han and Kaminski 2011, 2012; Hoyle et al. 2012; Trappe et al. 2012; observations of the authors). Both foliar and root up-take of methiozolin are suspected. Optimum annual bluegrass control is believed to be achieved through soil application where the herbicide is applied followed by irrigation in a similar manner as PRE-applied herbicides, which is also indicative of root absorption. These observations have led to the hypothesis that foliar absorption is minimal and phytotoxicity is primarily due to the herbicide contacting the soil followed by root absorption. The objectives of this research were to 1) determine if methiozolin has PRE activity against germinating annual bluegrass seed, 2) determine the relative importance of foliar versus root exposure in the phytotoxicity of POST-applied methiozolin, and 3) quantify absorption and translocation of foliar- and root- applied methiozolin using radio-tracer techniques.

## **Materials and Methods**

**General information.** Studies were conducted in a greenhouse located on the main campus of Auburn University in Auburn, AL (32.35°N, 85.29°W) between November 2011 and April 2012. The greenhouse was equipped with evaporative cooling panels that activated whenever temperatures exceeded 23 C. Plants received natural daylight only and photoperiods were approximately 10.7 and 13.3 hours for November and April, respectively. Relative humidity averaged to 68%. Annual bluegrass plants were grown from seed, which had been locally collected. All test plants were grown in 0.7 L pots with an 80 cm<sup>2</sup> soil surface area. Pots were fertilized (Miracle-Gro Water Soluble All Purpose Plant Food, Scotts Miracle-Gro Products,



Inc., Marysville, OH) every two weeks during establishment (28–8–16; 5.8 kg N ha<sup>-1</sup>); PRE and POST selective placement studies were also fertilized during the experiment.

All experiments were conducted as completely randomized designs with four replications per treatment and were repeated in time. All data were subjected to ANOVA using the GLIMMIX procedure in SAS (SAS® version 9.1.3, SAS Institute, Cary, NC). For all experiments, preliminary statistical analysis detected no interaction between treatments and experimental repetitions. Consequently, data were pooled across repetitions. Response variables were transformed where necessary as indicated to satisfy model assumptions. Presented means and confidence intervals were back-transformed where appropriate. LSmeans and adjusted 95% confidence intervals were used to detect significant differences between treatments; this method allows for multiple comparisons by providing family-wise error rate protection (Littell et al. 2006).

**Preemergence experiments.** Experiments were conducted to determine if methiozolin (MRC-01, Moghu Research Center, Daejeon, Korea) has PRE activity against germinating annual bluegrass seed. Indaziflam (Specticle 20 WSP, Bayer CropScience, Research Triangle Park, NC) inhibits cellulose biosynthesis and exhibits excellent annual bluegrass control when applied PRE, with some limited POST activity (Brosnan et al. 2012a; Meyers et al. 2009). Therefore, indaziflam was applied at 35 and 70 g ai ha<sup>-1</sup> in the same manner as methiozolin for comparison. Experiments consisted of a factorial arrangement of two soil types and two methiozolin rates. Soil types were native soil (Wickham sandy loam, pH 6.3) and United States Golf Association (USGA) root-zone (90:10 vol vol<sup>-1</sup> USGA grade sand to organic matter mix, pH 5.3). Methiozolin was applied at 1.68 and 3.36 kg ai ha<sup>-1</sup>. A nontreated check was also included with each soil type. All herbicide-containing treatments contained non-ionic surfactant at 0.25 % vol

vol<sup>-1</sup> (Activator 90, Dow AgroSciences LLC, Indianapolis, IN). Eight annual bluegrass seeds were scattered on the soil surface of each pot and pressed into the soil surface to ensure adequate seed-to-soil contact; the seeds were not buried. Treatments were applied using an enclosed spray cabinet, calibrated to deliver 280 L ha<sup>-1</sup>. Aboveground biomass was harvested 25 days after treatment (DAT) by cutting the foliage just above the crown, i.e. approximately 1.5 cm above the soil surface. Experiment data were transformed to percent reduction relative to the nontreated (i.e. control) within appropriate soil type for analysis (including ANOVA) and presentation.

**Postemergence selective placement experiments.** Experiments were conducted to evaluate foliar versus root exposure in the phytotoxicity of POST-applied methiozolin. Experiments included a factorial treatment arrangement of the two soil types previously described, two herbicides, two herbicide rates previously described, two annual bluegrass growth stages ('small,' approximately 2 tillers and 'large,' approximately 6 tillers, at the time of treatment), and three application methods. The three application methods were soil-only, foliar-only, and foliar-plus-soil. Each pot contained 5 plants. Foliar-only and foliar-plus-soil treatments were applied over-the-top in a conventional manner using the previously described sprayer. Foliar-only treatments were applied after covering the soil surface with 1 cm perlite. The perlite was removed 1 DAT. Soil-only treatments were applied by calculating the amount of spray solution that would contact the soil surface (assuming bare soil and 100% of the spray reaches the soil surface). This amount of spray solution was diluted in 10 mL of water and applied directly to the soil avoiding foliar contact. Overhead irrigation was withheld for 3 DAT. These methods are similar to previous experiments (McElroy et al. 2004a; McCurdy et al. 2009; Wehtje and Walker 2002; Williams et al. 2003).

Aboveground biomass was harvested 25 DAT in the same manner as PRE experiments. Additionally in POST experiments, aboveground biomass (i.e. regrowth) was harvested again 14 d later (39 DAT). Statistical analysis of POST data indicated that an arcsine square root transformation of the response variable best satisfied model assumptions. Experiment data were transformed to percent reduction relative to the nontreated (i.e. control) within the appropriate soil type and growth stage.

**Absorption and translocation of foliar-applied methiozolin.** Individual annual bluegrass plants were grown in individual containers in native soil (previously described) to approximately the 3-tiller growth stage. Treatment solution was prepared to simulate a 2.24 kg methiozolin ha<sup>-1</sup> application rate in 187 L ha<sup>-1</sup> carrier volume. Treatment solution was prepared by combining <sup>14</sup>C-methiozolin and nonlabeled methiozolin such that the total concentration was 11.98 mg mL<sup>-1</sup> and the radioactive concentration was 0.084 MBq mL<sup>-1</sup>. A single ~5 µL drop treatment solution was applied to a fully mature leaf, approximately half-way between the leaf base and tip. Fourteen plants were treated in this manner. Four treated plants were randomly selected for harvest 24, 48, and 72 hours after treatment (HAT). At harvest, the treated leaf was detached from the plant and the ~1 cm section on which the drop had been placed, i.e. the target area, was excised. The leaf target area was placed into a 20 mL scintillation vial along with 1 mL of a water-methanol (1:1; v:v) solution. The excised leaf target area was agitated with a swirling motion for 1 min to remove any unabsorbed radioactivity. Leaf target area tissue was then removed, and 10 mL of scintillation fluid (Scinti-safe, ThermoFisher Scientific Inc., Pittsburg, PA) was added to the vial in preparation for scintillation spectrometry. The two remaining portions of the treated leaf and the remainder of the plant were partitioned into treated leaf target area, treated leaf above target area, treated leaf below target area, remainder of foliage on treated

tiller, foliage of adjacent tillers, crown, and roots. All plant tissue samples were dried at 45 C (24 H), combusted in a biological oxidizer (OX-700, R. J. Harvey Instrument Corp., Tappan, NY), and radioactivity was quantified through scintillation spectrometry. Radioactivity detected in the leaf wash and tissue sections was expressed as the percent relative to the amount applied. Total recovery was  $\geq 98\%$  of amount applied. The experiment included four single plant replicates for each of the three harvest times. These methods are similar to previous experiments (Wilcut, et al. 1989; Williams et al. 2003). Statistical analysis indicated that a log transformation of the response variable best satisfied model assumptions.

**Absorption and translocation of root-applied methiozolin.** Individual annual bluegrass plants were grown to the 3-tiller stage as previously described. Fourteen plants were removed from their containers and the roots washed free of soil. Plants were placed in hydroponic culture (not aerated) for 2 d prior to treatment application. An aqueous solution was prepared using  $^{14}\text{C}$ -labeled and nonlabeled methiozolin such that the total concentration and radioactive concentration was  $5.0 \mu\text{g mL}^{-1}$  (ppm) and  $415.0 \text{ MBq mL}^{-1}$ , respectively. The 5.0 ppm concentration was selected since it represents a  $2.24 \text{ kg ha}^{-1}$  application rate, with the methiozolin becoming incorporated to a 3 cm depth, and only 19% of the amount that would enter the soil remaining available within the soil solution at field capacity [unpublished data of the authors using established methods (Adams et al. 1982; Wehtje et al. 2000)]. Plants were placed in this solution such that the roots remained continually submerged. Four plants were randomly selected and removed 24, 48, and 72 HAT. Roots were washed twice in 100 mL water at room temperature for 30 s by swirling motion to remove any unabsorbed  $^{14}\text{C}$ -methiozolin. Plants were partitioned into foliage, crown, and roots. Plant tissues were dried as previously described, weighed, and combusted as previously described. The amount of methiozolin

equivalents per gram plant tissue was then determined. The experiment included four, single-plant replicates for each of the three harvest times. These methods are similar to previous experiments (Wilcut, et al. 1989; Williams et al. 2003). Statistical analysis indicated that a log transformation of the response variable best satisfied model assumptions.

**Autoradiograms.** Autoradiograms are effective for the visualization of herbicide translocation (Wehtje et al. 2007). The two additional plants that were treated in both the foliar-applied and the root-applied radio-tracer experiments were used for preparation of autoradiograms. Plants were harvested 72 HAT and unabsorbed herbicide was removed as previously described. In lieu of preparation for combustion, plants were left intact, pressed, and dried at 35 C for 1 wk. Autoradiograms were prepared using phosphorescence imaging techniques as described by Wehtje et al. (2006). Images presented were chosen on the basis of image quality and clarity.

## Results and Discussion

**Preemergence experiments.** Methiozolin and indaziflam resulted in complete annual bluegrass control [100%; 95% confidence interval (CI95) 94 to 106%] (Table 1). Control was not affected by either soil type, herbicide, or herbicide rate ( $P > 0.05$ ), indicating that the only significant factor was whether a herbicide was applied or not. These data indicate that methiozolin at 1.68 kg ha<sup>-1</sup> or greater is an effective PRE control option for annual bluegrass. Effective PRE annual bluegrass control from methiozolin has been previously reported (Hwang and Koo 2009; Nam et al. 2012). Nam et al. (2012) reported that the *S*-isomer of methiozolin resulted in excellent PRE annual bluegrass control while the *R*-isomer had no noticeable herbicidal activity. Longevity of methiozolin in the field is required for adequate, season-long control, which may be an issue. Norsworthy et al. (2011) reported that methiozolin PRE activity on barnyardgrass ceased 6 wk

after application. Hwang and Koo (2009) previously found no difference in PRE control with soil texture (i.e. loam sand, sandy loam, or clay loam), which agrees with this research. These data also indicate that indaziflam at 35 g ai ha<sup>-1</sup> or greater is an effective herbicide for PRE annual bluegrass control. Previous research also reports 93 to 100% PRE annual bluegrass control with indaziflam at 30 to 60 g ai ha<sup>-1</sup> in Texas 28 weeks after treatment (Brosnan et al. 2012a).

**Postemergence selective placement experiments.** *Annual bluegrass control 25 DAT.* POST selective placement controlled annual bluegrass  $\leq 50\%$  (Table 2). Growth stage and herbicide were the only significant parameters, indicating that herbicide rate, soil-type, and treatment placement did not influence annual bluegrass control. Both herbicides resulted in better control of the small growth stage compared to the large growth stage. Methiozolin controlled the small growth stage 50% and the large growth stage 28%. While indaziflam controlled the small growth stage 32% and the large growth stage 23%.

*Annual bluegrass control 39 DAT.* Annual bluegrass control 39 DAT was generally greater than 25 DAT, but maximum control remained  $< 80\%$  (Table 3). Similar to 25 DAT, differences between growth stages were detected 39 DAT; the smaller growth stage was controlled greater than the larger growth stage. Additionally, differences between treatment placement and methiozolin rate parameters were detected. This increase in observed control is likely due to the harvest at 25 DAT and subsequent regrowth period.

ANOVA indicated that soil type and interactions with soil type were not significant for annual bluegrass control. Data were pooled across soil type for subsequent analysis and presentation. This finding agrees with that of 25 DAT data. Hwang and Koo (2009) previously found no differences in POST control with soil texture (loam sand, sandy loam, or clay loam).

Brosnan et al. (2012a) also reported similar annual bluegrass control between soil-based (silt loam) and sand-based root-zones. A significant herbicide main effect was detected, so subsequent analysis was conducted separately for each herbicide.

Methiozolin resulted in 55% annual bluegrass control (CI95 51 to 58%) across rate, growth stage, and treatment placement (data not shown). Annual bluegrass response to POST applied methiozolin had significant effects ( $P < 0.03$ ) including treatment placement, growth stage, methiozolin rate, and treatment placement by growth stage interaction. Annual bluegrass control from the high rate ( $3.36 \text{ kg ai ha}^{-1}$ ) was 61% (CI95 54 to 67%) while control from the low rate ( $1.68 \text{ kg ai ha}^{-1}$ ) was only 48% (CI95 42 to 55%) as averaged across treatment placement and growth stage (data not shown). Han and Kaminski (2011) reported near complete annual bluegrass control from  $2.0 \text{ kg methiozolin ha}^{-1}$ ; however, one perennial biotype only exhibited moderate injury. The smaller stage was controlled better (65%; CI95 58 to 71%) than the larger stage (44%; CI95 38 to 50%) across rate and treatment placement. Hwang and Koo (2009) reported 80 and 60% growth inhibition of two- and four-leaf stage annual bluegrass from methiozolin applied at  $0.50 \text{ kg ha}^{-1}$ . Foliar-plus-soil treatment placement resulted in the greatest annual bluegrass control for each rate and growth stage, although not necessarily statistically significant (Table 3). Across rate and growth stage, foliar-plus-soil application placement resulted in 64% control (CI95 57 to 71%), which was similar to the control resulting from foliar-only treatment (53%; CI95 45 to 61%). However, control resulting from foliar-plus-soil application was superior to soil-only application, i.e. 45% control (CI95 37 to 54%). Overall, higher methiozolin rate, smaller growth stage, and foliar-plus-soil application resulted in the best annual bluegrass control (Table 3). Previous research reported that soil-only and foliar-plus-soil applied methiozolin ( $1.0 \text{ kg ha}^{-1}$ ) treatment placement resulted in greater annual bluegrass

control compared to foliar-only treatment placement (Brosnan et al. 2012a). Current indications are that annual bluegrass control in the field requires multiple applications and complete control may still not be achieved (Brosnan et al. 2012a; Haguewood et al. 2012; Han and Kaminski 2011, 2012; Hoyle et al. 2012; Trappe et al. 2012; observations of the authors). However, this research was limited to a single application.

Indaziflam resulted in 27 to 56% annual bluegrass control 39 DAT (Table 4). Annual bluegrass response to POST applied indaziflam had significant effects ( $P < 0.034$ ) including treatment placement and treatment placement by growth stage interaction. Indaziflam controlled the small growth stage of annual bluegrass best (56%) from soil-only application compared to foliar-plus-soil and foliar-only treatment placements (Table 4). No differences in annual bluegrass control were detected when applied to the large growth stage; control was  $< 50\%$ . Previous research also reports that indaziflam has reduced POST efficacy for annual bluegrass control relative to the PRE timing. Brosnan et al. (2012a) reported 96 to 100% annual bluegrass control 30 weeks after treatment from indaziflam at  $35 \text{ g ai ha}^{-1}$  in Tennessee and Georgia but control was reduced to 51 to 88% when application was delayed for eight weeks (early POST timing).

**Absorption and translocation of foliar-applied methiozolin.** ANOVA indicated that the tissue sampled was a significant effect, ( $P < 0.001$ ), but time after treatment was not significant ( $P = 0.122$ ). However, time after treatment is presented in Table 5 to show trends. This indicates that foliar methiozolin absorption and translocation is likely complete 24 hours after application.

Methiozolin was absorbed by the foliage; maximum absorbance was 55% of the amount applied (Table 2). Lipophilic herbicides are known to readily absorb into plant foliage (Briggs et al. 1982; Hsu et al. 1990). Other reports of POST applied herbicides to annual bluegrass foliage



found different absorption amounts. Flazasulfuron ( $\log K_{ow} = -0.06$ ) was reported to have 95% absorption 72 HAT (Brosnan et al. 2010; Senseman 2007). Ethofumesate ( $\log K_{ow} = 2.7$ ) absorption averaged 33% from treatment until 14 DAT, with a maximum absorption of 42% (Kohler and Branham 2002; Senseman 2007). Mesotrione ( $\log K_{ow}$  unknown) was reported to have approximately 48% absorption (Goddard 2009; Senseman 2007). Bispyribac ( $\log K_{ow} = -1.03$ ) reached maximum absorption of 29%, 24 HAT (Lycan and Hart 2006; Senseman 2007). Lastly, 83% of applied prohexadione calcium (calcium salt of 3,5-dioxo-4-propionylcyclohexane-carboxylic acid;  $K_{ow}$  unknown) was absorbed by annual bluegrass foliage (Beam and Askew 2007).

Methiozolin translocation was greatest above the target area (10% of applied). Conversely, only 1.4% of the applied methiozolin was detected in the target leaf below the target area, and only 1.3% (total of all other sections) of the applied methiozolin was detected in the plant beyond the target leaf. These data indicate that methiozolin translocation occurs upward in the plant, toward the leaf tip with negligible basipetal translocation. Therefore, it is likely that methiozolin translocates in the xylem with the transpirational stream and unlikely that methiozolin translocates in the phloem, which is also consistent with its  $\log K_{ow}$  value of 3.9 (Kleier 1988). Other reports of translocation following annual bluegrass foliar exposure are as follows: Ethofumesate was also found to have essentially no translocation from the treated, mature leaf (Kohler and Branham 2002). Bispyribac has also been reported to have little translocation out of the treated leaf of annual bluegrass; only 9.3% of absorbed bispyribac was detected beyond the treated leaf, compared to 2.6% of absorbed methiozolin (Lycan and Hart 2006). Goddard (2009) reported approximately 20% translocation of mesotrione out of the treated leaf. Prohexadione was reported to translocate 11% of applied to the roots and 15% of

applied to the foliage beyond the treated leaf (Beam and Askew 2007). These findings suggest that foliar-only application of methiozolin may be ineffective due to minimal translocation to the remainder of the plant.

Autoradiograms are in general agreement with data obtained through  $^{14}\text{C}$ -methiozolin quantification (Figure 2). The autoradiogram indicates a strong concentration/absorption at the target area, limited translocation toward the leaf tip, and negligible translocation to the rest of the plant.

**Absorption and translocation of root-applied methiozolin.** ANOVA indicated there were significant differences in the  $^{14}\text{C}$  found in the different tissues ( $P < 0.001$ ) and the amount of total  $^{14}\text{C}$  in the plants over time ( $P = 0.004$ ). However, there was no tissue by time interaction ( $P = 0.545$ ).

Roots readily absorbed methiozolin; however translocation from the roots to the foliage was minimal; concentration in the foliage was  $< 8 \mu\text{g methiozolin g}^{-1}$  (Table 6). Methiozolin translocation to the crown was  $\leq 30 \mu\text{g methiozolin g}^{-1}$ , indicating that methiozolin is absorbed by the roots and is moderately translocated to the crown of annual bluegrass. This absorption and translocation pattern is consistent with the  $\log K_{ow}$  value for methiozolin (3.9). Herbicides with  $\log K_{ow}$  values in this range have excellent absorption due to their membrane permeation, but are not well translocated in the xylem due to their low water solubility (Briggs et al. 1982; Hsu et al. 1990). Previous research on annual bluegrass root absorption and translocation of mesotrione reported approximately 30% absorption and only 5% translocation to the foliage (Goddard 2009). 77% of root absorbed bispyribac translocated to the shoots 72 HAT (Lycan and Hart 2006). These results are in comparison to 17.6% of methiozolin in the current study 72 HAT.

This finding suggests that methiozolin application to the soil, where it can be absorbed by the roots, may result in effective control due to root and shoot (via moderate translocation) exposure.

Time after treatment, while significant ( $P = 0.004$ ), only accounted for 1.1% of the variation explained by the model while tissue sample accounted for 95.3% of the variation explained by the model (data not shown). Contrast statements exploring the time after treatment indicated that 48 and 72 HAT were similar ( $P = 0.649$ ). These data suggest that methiozolin absorption and translocation from the roots is nearly complete 24 HAT. Absorption and translocation was complete 48 HAT. These figures largely agree with foliar absorption and translocation.

Autoradiograms are in general agreement with data obtained through  $^{14}\text{C}$ -methiozolin quantification (Figure 2). The autoradiogram indicates a strong concentration/absorption at the roots, moderate translocation into the crown, and very limited translocation to the foliage. This is again indicative of the  $\log K_{ow}$  value for methiozolin (3.9), which suggests excellent root absorption, but difficulty in translocation due to poor partitioning into the xylem sap (Briggs et al. 1982; Hsu et al. 1990). As with foliage treatment, the autoradiogram of  $^{14}\text{C}$ -methiozolin treated roots provides qualitative support to our quantitative findings.

**Implications.** These data suggest that methiozolin applied PRE can effectively control annual bluegrass. However, longevity of methiozolin in the field is required for adequate, season-long control, which may be an issue (Norsworthy et al. 2011). POST applied methiozolin is more effective at higher rates and smaller growth stages. Foliar-plus-soil methiozolin application trended to result in the best control, compared to either foliar-only or soil-only applications. Previous research reported foliar-plus-soil and soil-only application resulted in greater control than foliar-only application (Brosnan et al. 2012a).

Future research on methiozolin metabolism is necessary to corroborate data to parent methiozolin molecule translocation. However, absorption and translocation data indicate that methiozolin is absorbed by both the leaves and roots and moderately translocates arcopetally, presumably through the xylem. While current data are limited, a successful methiozolin application in the field likely requires both root and foliar exposure, due to limited translocation. Due to the fact that control is limited from a single methiozolin application (as observed in POST experiments), successful field application of methiozolin requires multiple, timely applications directed toward the roots and foliage of annual bluegrass.

Table 1. Preemergence (PRE) annual bluegrass control from methiozolin and indaziflam in two combined greenhouse experiments in Auburn, AL in 2011 to 2012.

Herbicide <sup>a</sup>	Control <sup>b</sup>	95% confidence limits	
		Lower	Upper
		-----%-----	
indaziflam	100.0	94.0	105.9
methiozolin	100.0	94.0	105.9
nontreated	0.0	-8.4	8.4

<sup>a</sup> Herbicide rates applied were 1.68 and 3.36 kg methiozolin ha<sup>-1</sup> and 35 and 70 g indaziflam ha<sup>-1</sup>. Data were pooled across rates as no differences were detected.

<sup>b</sup> Control measured as percent reduction in aboveground biomass relative to the nontreated at 25 days after treatment (DAT).

Table 2. Postemergence (POST) annual bluegrass control 25 days after treatment (DAT) in two combined greenhouse experiments in Auburn, AL in 2011 to 2012.

Herbicide <sup>a</sup>	Growth stage	Control <sup>b</sup>	95% confidence limits	
			Lower	Upper
			-----%-----	
Methiozolin	2 tiller	50 A	44	56
	6 tiller	28 B	24	34
Indaziflam	2 tiller	32 a	27	38
	6 tiller	23 b	19	28

<sup>a</sup> Herbicide rates applied were 1.68 and 3.36 kg methiozolin ha<sup>-1</sup> and 35 and 70 g indaziflam ha<sup>-1</sup>. Data were pooled across rates as no differences were detected.

<sup>b</sup> Control measured as percent reduction in aboveground biomass relative to the nontreated. Means sharing a letter are not significantly different according to a back transformed 95% confidence interval about the mean within parameter.

Table 3. Postemergence (POST) annual bluegrass control from methiozolin as influenced by rate, growth stage, and treatment placement in two combined greenhouse experiments in Auburn, AL in 2011 to 2012.<sup>a</sup>

Treatment			Control <sup>b</sup>	95% confidence limits	
Rate	Growth stage	Placement		Lower	Upper
kg ha <sup>-1</sup>			-----%-----		
1.68	2 tiller	Soil-only	56 AB	35	77
		Foliar-plus-soil	63 A	49	76
		Foliar-only	56 AB	40	71
	6 tiller	Soil-only	27 B	15	41
		Foliar-plus-soil	49 AB	37	62
		Foliar-only	39 AB	29	50
3.36	2 tiller	Soil-only	73 a	41	104
		Foliar-plus-soil	77 a	56	98
		Foliar-only	61 ab	40	81
	6 tiller	Soil-only	27 b	12	44
		Foliar-plus-soil	65 a	50	78
		Foliar-only	57 a	42	71

<sup>a</sup> Evaluated 39 days after treatment (DAT); data pooled over soil types.

<sup>b</sup> Control measured as percent reduction in aboveground biomass relative to the nontreated. Means within a common rate sharing a letter are not significantly different according to the back transformed 95% confidence interval within growth stage.

Table 4. Postemergence annual bluegrass control from indaziflam as influenced by growth stage and treatment placement in two combined greenhouse experiments in Auburn, AL in 2011 to 2012.<sup>a</sup>

Treatment		Control <sup>b</sup>	95% confidence limits		
Growth stage	Placement		Lower	Upper	
		-----%-----			
2 tiller	Soil-only	56 A	46	66	
	Foliar-plus-soil	35 B	27	44	
	Foliar-only	27 B	18	36	
6 tiller	Soil-only	39 a	32	47	
	Foliar-plus-soil	44 a	37	51	
	Foliar-only	47 a	40	53	

<sup>a</sup> Indaziflam applied at 35 and 70 g ai ha<sup>-1</sup>; data pooled across rates as no differences were detected (P = 0.748).

<sup>b</sup> Control measured as percent reduction in aboveground biomass relative to the nontreated at 39 days after treatment. Means sharing a letter are not significantly different according to the back transformed 95% confidence interval within growth stage.



Table 5. Foliar absorption and translocation of  $^{14}\text{C}$ -methiozolin by annual bluegrass over time from two combined laboratory experiments.<sup>a</sup>

Sample	Time after treatment (hours) <sup>b</sup>			Mean <sup>c</sup>		95% confidence limits	
	24	48	72			Lower	Upper
	-----% of applied-----						
Leaf wash (unabsorbed methiozolin)	54.4	44.3	37.8	45.0	A	36.6	55.3
Treated leaf target area	30.2	38.5	43.7	37.0	AB	30.1	45.5
Treated leaf above target	7.9	10.4	12.3	10.0	C	8.2	12.3
Treated leaf below target	1.7	1.4	1.2	1.4	D	1.2	1.8
Remainder of foliage on treated tiller	0.9	0.9	0.8	0.8	E	0.7	1.0
Remainder of shoots	0.2	0.3	0.3	0.2	F	0.2	0.3
Crown	0.2	0.3	0.2	0.2	F	0.2	0.2
Roots	0.1	0.1	0.1	0.1	F	0.1	0.2

<sup>a</sup> Leaf spotting was a 5  $\mu\text{L}$  droplet of methiozolin corresponding to a 2.24 kg ha<sup>-1</sup> rate.

<sup>b</sup> Time after treatment not significant in ANOVA ( $P = 0.122$ ) but presented to show trends in data.

<sup>c</sup> Means sharing a letter are not significantly different according to the adjusted 95% confidence interval.

Table 6. Root absorption and translocation of <sup>14</sup>C-methiozolin by annual bluegrass over time in two combined laboratory experiments.<sup>a</sup>

Time after treatment <sup>b</sup> hours	Tissue Sample	Mean <sup>c</sup>	95% confidence limits		
			Lower	Upper	
		-----µg methiozolin per g plant tissue-----			
24	Foliage	4.3 C	3.2	5.8	
	Crown	23.8 B	17.7	32.0	
	Root	147.3 A	109.6	198.1	
48	Foliage	6.7 C	5.2	8.5	
	Crown	28.1 B	22.0	35.8	
	Root	188.6 A	148.1	240.2	
72	Foliage	7.7 C	6.1	9.7	
	Crown	30.0 B	23.8	37.9	
	Root	177.0 A	140.1	223.5	

<sup>a</sup> Solution contained 5 ppm methiozolin corresponding to a 2.24 kg ha<sup>-1</sup> rate.

<sup>b</sup> Contrast statements did not detect differences between 48 and 72 hours after treatment (P = 0.649).

<sup>c</sup> Means sharing a letter within time after treatment are not significantly different according to the adjusted 95% confidence interval.

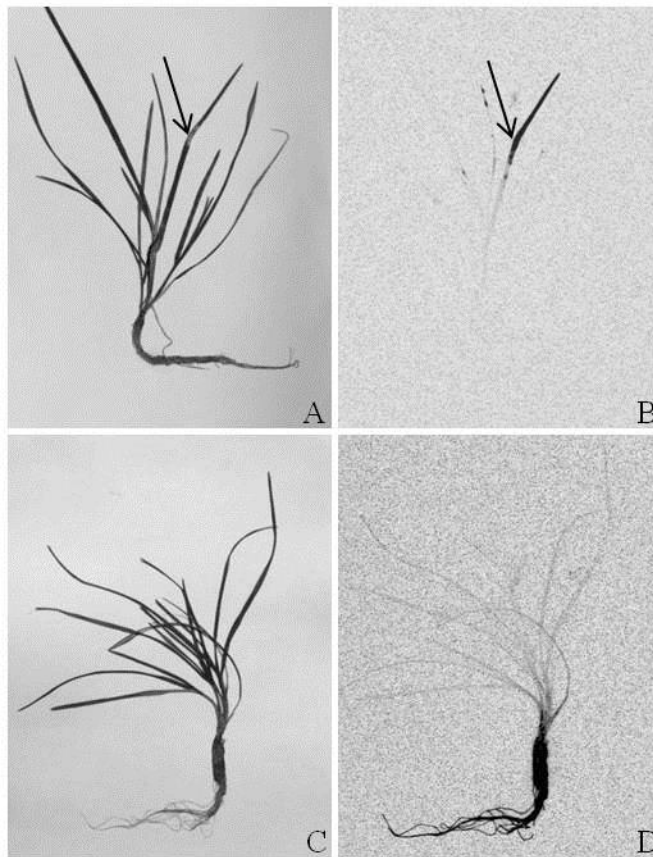


Figure 2. Annual bluegrass 72 hours after treatment with  $^{14}\text{C}$ -methiozolin. Rate simulated was  $2.24 \text{ kg ha}^{-1}$  for foliar and root application. (A) Plant which received foliar application. Arrow indicates leaf spotting location. (B) Autoradiogram of A, darkness indicates greater abundance of  $^{14}\text{C}$ -methiozolin. (C) Plant which was root exposed via spiked hydroponic solution. (D) Autoradiogram of C, darkness indicates greater abundance of  $^{14}\text{C}$ .

## **Methiozolin Sorption and Mobility in Sand-Based Root-Zones.**

[This chapter has been accepted pending revision for publication as Flessner, M. L., G. R.

Wehtje, J. S. McElroy, and J. A. Howe. 2014. Methiozolin sorption and mobility in sand-based root-zones. *Pest. Manag. Sci. Provisionally accepted*]

### **Introduction**

Methiozolin (5-(2,6-difluoro-benzyloxymethyl)-5-methyl-3-(3-methyl-thiophen-2-yl)-4,5-dihydro-isoxazole) (Figure 1) is a herbicide of the isoxazoline class (Hwang et al. 2005). Methiozolin selectively controls annual bluegrass (*Poa annua* L.) in creeping bentgrass (*Agrostis stolonifera* L.) and hybrid bermudagrass (*Cynodon dactylon* × *C. transvaalensis* Burt-Davy) (Brosnan et al. 2013a; Flessner et al. 2013; Hwang and Koo 2009; Koo et al. 2010; Koo et al. 2014; McCullough and Gómez de Barreda 2012; McCullough et al. 2013). Currently, there are few options for selective annual bluegrass control in these turfgrasses, especially in golf course putting greens. Thus, methiozolin is a desirable candidate for use in golf courses, where it is already registered for use in South Korea. Registration is currently being pursued in the United States for use on golf course putting greens.

Methiozolin reportedly has both pre-emergent (PRE) and post-emergent (POST) herbicidal activity on annual grass weeds (Brosnan et al. 2013a; Flessner et al. 2013; McCullough et al. 2013). Grossmann et al. (2012) reported that it inhibited tyrosine aminotransferase (TAT), which blocks the production of plastoquinone and tocopherol, both of

which are important in membrane protection to oxidative stresses. However, TAT inhibition research was conducted using nongraminaceous plants (i.e., *Lemna paucicostata* L. and *Arabidopsis thaliana* L.), and thus may have a different mode-of-action in grasses (Grossmann et al. 2012). Another reported mode-of-action for methiozolin is cell wall biosynthesis inhibition, which was evaluated using maize (*Zea mays* L.) (Lee et al. 2007). However, the exact site or enzyme of inhibition is believed to be different from that of other cell wall biosynthesis inhibiting herbicides (Hwang et al. 2005; Lee et al. 2007; Nam et al. 2012).

Methiozolin is absorbed by the roots and foliage of annual bluegrass (Brosnan et al. 2013a; Flessner et al. 2013; McCullough et al. 2013). Root exposure and subsequent absorption is required for POST control (Brosnan et al. 2013a; Flessner et al. 2013; Koo et al. 2014). Koo et al. (2014) reported that no herbicidal activity resulted from leaf-only application. Therefore, methiozolin must be available for root uptake in the soil to effectively control weeds in the field. Herbicide sorption to soil particles renders it unavailable for root uptake, but high solubility and lack of charge can cause leaching from the root-zone. Thus, herbicide efficacy is greatest if sorption and mobility are intermediate. Little has been reported about the soil sorption and mobility of methiozolin. Hwang and Koo (2009) reported no differences in PRE annual bluegrass control with soil texture (i.e., loam sand, sandy loam, or clay loam). Similar POST annual bluegrass control (Brosnan et al. 2013a) and creeping bentgrass injury (Brosnan et al. 2013b) has been reported between silt loam and sand-based medias. However differences in creeping bentgrass root-length density were observed between sand- and soil-based medias (Brosnan et al. 2013b).

Herbicide water solubility and octanol-water partitioning coefficient ( $K_{ow}$ ) are predictive of soil sorption and mobility. Methiozolin has a water solubility of  $3.4 \text{ mg L}^{-1}$  and a  $\log K_{ow}$  value

of 3.9, indicating that it is hydrophobic (Koo et al. 2010; Anonymous 2012b). Methiozolin does not have an ionizable functional group (Figure 1), and therefore is not readily affected by pH or electrostatic interactions with soil colloids.

Soil sorption of herbicides is strongly influenced by soil physical parameters, primarily organic matter and clay content. Studies indicate that as organic matter increases, herbicide sorption increases (Harris 1966; Harris and Sheets 1965; Nearpass 1965; Obrigawitch et al. 1981; Stougaard et al. 1990; Talbert and Fletchall 1965; Upchurch and Mason 1962; Weber et al. 1969). This relationship can be due to hydrophobic interactions between the herbicide and organic matter or to sorption of charged herbicide molecules to various sites within organic matter. Clay, which is typically negatively charged, sorbs positively-charged molecules and repels negatively-charged molecules. Soil texture also influences herbicide-soil interactions. (Goetz et al. 1989; Helling 1971; Patterson et al. 1982; Tisdale et al. 1985). Shea (1989) concluded that pesticides have greater attraction to organic matter compared to clay particles due to more interaction sites.

Sorption to soil also influences herbicide soil mobility. As a herbicide becomes sorbed to soil colloids, it generally becomes less mobile in the soil (Best and Weber 1974; Helling 1971; Ladlie et al. 1976a, b; Stougaard et al. 1990). Uncharged herbicides with high water solubility and low  $K_{ow}$  tend to be poorly sorbed to soil and are therefore more mobile due to their greater affinity for the water phase. Hydrophobic herbicides with high  $K_{ow}$  values such as methiozolin tend to sorb to soil rather than stay in soil solution and are therefore less mobile (Ross and Lembi 1999; Sakaliene et al. 2007). However, herbicides that are charged may not adhere to these trends. Glyphosate, which typically exists as a cation or zwitterion, has high water solubility but sorbs to soils due to electrostatic attraction (Al-Rajab et al. 2008; Sprankle et al. 1975).

Understanding methiozolin sorption and mobility in soil is an important step toward evaluation of the environmental and agronomic consequences of applying this herbicide to control weeds. Herbicides with high soil mobility may quickly leach, potentially resulting in reduced herbicide efficacy, off-target effects, and ground and surface water contamination (Mueller and Banks 1991). The objectives of this research were to evaluate methiozolin for sorption and mobility in soil with particular interest to sand-based media commonly used in golf course putting greens.

### **Materials and Methods**

**Soils.** Nine rooting media (herein termed ‘soil’) were evaluated. Sand meeting the particle size and content specifications for United States Golf Association (USGA) root-zone construction was used alone and in combination with peat (Sphagnum peat moss; Sta-Green® Enhanced Canadian Sphagnum Peat Moss; Majestic Earth, Agawam, MA) at 2.5, 5.0, 10, and 20% dry wt wt<sup>-1</sup>. Additionally, three soils were collected from putting greens meeting the USGA specifications that varied in age from newly constructed (acquired from Butler Sand Co., Butler GA), to established for 10 years, to established > 15 years. The 10 and > 15 year old soils were collected from greens (constructed as 80:20 USGA sand: reed sedge peat) at the Auburn University Turfgrass Research Unit in Auburn, AL (32.29°N, 85.53°W). These soils were collected from 4 to 18 cm depth. Lastly, a ‘field soil’ was collected from Auburn University’s Plant Breeding Unit near Tallassee, Alabama (32.34°N, 85.29°W). This soil is a sandy loam from the Wickham sandy loam series (Fine-loamy, mixed, semiactive, thermic Typic Hapludults) collected from the soil surface to 25 cm depth after cultivating to a 30 cm depth. All soils were air-dried, passed through a 2-mm sieve, and homogenized. Hwang et al. (2013) reported the soil

half-life of methiozolin to be approximately 49 days under aerobic conditions, but a half-life could not be estimated under anaerobic, flooded conditions in laboratory due to limited degradation. Thus, soils were not autoclaved or treated to prevent microbial activity since the experiments were conducted under saturated conditions. Soil physical and chemical properties are reported in Table 7. Soil pH was determined by 1:1 soil:deionized water mixture (Soil Survey Staff, 2004). Organic matter content was determined via combustion (Elementar, Elementar Americas, Inc.) according to the methods of Yeomans and Bremner (1991) and Baldock and Nelson (2000). Soil textural analysis was conducted using the hydrometer method (Soil Survey Staff, 2004).

**Chemicals.** Benzyl- $^{14}\text{C}$ -methiozolin ( $> 99\%$  purity;  $4.57 \text{ MBq mg}^{-1}$ ) was provided by the Moghu Research Center (Daejeon, Korea). For batch equilibrium studies, an experimental solution was prepared by combining  $\text{CaCl}_2$ ,  $^{14}\text{C}$ -methiozolin, and formulated methiozolin (PoaCure®; Moghu Research Center) such that the  $\text{CaCl}_2$  concentration was  $0.01 \text{ M}$ , the total methiozolin concentration was  $1 \text{ mg L}^{-1}$ , and the radioactive concentration was  $\sim 83 \text{ Bq mL}^{-1}$ . For soil thin-layer chromatography studies, spotting solution used was a 1:1 water:methanol ( $\text{vol vol}^{-1}$ ) solution with  $^{14}\text{C}$ -methiozolin added such that the radioactivity was  $\sim 580 \text{ Bq mL}^{-1}$ .  $^{14}\text{C}$ -isoxaben ( $> 98\%$  purity;  $0.70 \text{ MBq mg}^{-1}$ ; Ag-tracers, Dow Chemical, Midland, MI) solution was similarly prepared for soil thin-layer chromatography studies. The labeled carbon for isoxaben was the pyrimidine-2-carbon. Isoxaben was included in mobility studies for comparison of methiozolin to a similar mode-of-action (Heim et al. 1990; Lee et al. 2007; Senseman 2007).

**Sorption.**



*Kinetics.* Preliminary experiments were conducted to determine methiozolin soil sorption over time in order to evaluate the 48 hours equilibration time used in subsequent sorption and desorption studies. The field soil and newly constructed USGA sand were evaluated using batch equilibrium methods, similar to other studies (Goetz et al. 1986; Patterson et al. 1982; Oliveira et al. 2011). Sorption was evaluated in triplicate in the two soil samples at 6, 12, 24, and 48 hours. For each sample, 5 g of each soil were put in 25 mL glass test tubes with 15 mL methiozolin solution (i.e., 1 mg L<sup>-1</sup>). Test tubes were sealed with aluminum foil lined caps, mixed on a vortex shaker for 30 sec, and then placed on a mechanical shaker for their allotted time. Tubes were removed from the mechanical shaker and centrifuged for 15 min at 1400 g to separate soil particles from the supernatant. Two, 1 mL aliquots of supernatant from each test tube were removed, combined with 10 mL scintillation fluid (Universol, MP Biomedicals, Solon, OH), and analyzed for radioactivity. Radioactivity was quantified via liquid scintillation spectroscopy corrected to disintegrations per minute (dpm) within the device (LS 6500 Multi-Purpose Scintillation Counter). An average of two subsamples was used to determine the amount of methiozolin left in solution (not sorbed to the soil particles) for each replicate sample. Soil sorption coefficients (K<sub>d</sub> values) were determined from this average. K<sub>d</sub> values were calculated using the following equation:

$$K_d = (\mu\text{g methiozolin adsorbed g}^{-1} \text{ soil}) \div (\mu\text{g methiozolin ml}^{-1} \text{ solution}) \quad [1]$$

The experiment was repeated in time to achieve two experimental replications.

*Sorption.* Soil sorption studies were conducted on all soils using batch equilibrium methods, previously described with minor changes as noted subsequently. Based on kinetic experiments, a 48 hour equilibration time was used on the mechanical shaker. After equilibration, samples were centrifuged (2.5 hours at 1400 g) to separate soil particles from the supernatant. In addition to K<sub>d</sub>

values, organic carbon sorption coefficients ( $K_{oc}$ ) were calculated from  $K_d$  values using the following equation:

$$K_{oc} = (K_d) \div (\text{fraction organic carbon}) \quad [2]$$

where organic carbon was adjust from organic matter from the equation:

$$\% \text{ organic matter} = 1.7 (\% \text{ organic carbon}) \quad [3]$$

(Strebe and Talbert 2001; Weber 1995). The percentage of methiozolin remaining in solution (not sorbed to soil) was also calculated. A test tube with no soil was included to assess possible sorption to the test tube walls and test tube cap. Recovery of  $^{14}\text{C}$  from these check tubes was > 96%. Sorption experiments were conducted as a completely randomized design with 4 replications per treatment, and the entire experiment was repeated in time. The amount of methiozolin added initially, minus the amount of methiozolin detected in the solution is assumed to be sorbed to the soil and therefore not immediately available for plant uptake.

*Effect of pH.* The influence of soil pH was investigated with the field soil and the newly constructed USGA sand that were adjusted to a range of pH values. Actual pH values were 4.72, 6.02, 6.87 and 7.22 for the field soil and 5.03, 6.02, 6.77, and 7.28 for the USGA sand. Soil pH adjustment was made with agricultural grade lime (90% calcium carbonate equivalent) and iron sulfate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) as necessary. Several pH measurements were taken through time to ensure that pH had equilibrated before experiment initiation (data not shown). These soils were then analyzed using the batch equilibrium method previously described. Experiments were conducted as a completely randomized design with 4 replications per treatment and the entire experiment was repeated in time.

**Desorption by Dilution.** Desorption studies were conducted for all soils used in sorption studies, but only those that had not be adjusted for pH were evaluated. Methodology was based on a study by Oliveira et al. (2011). Desorption studies were conducted immediately following soil sorption studies by removing an additional 1 mL aliquot of supernatant in addition to the two aliquots (1 mL each) removed for radioactivity quantification in the sorption experiment. The supernatant that was removed (i.e., 3 mL total) was replaced with 3 mL 0.01 M CaCl<sub>2</sub> solution. Soil solutions were mixed using a vortex mixer for 30 sec then placed on a mechanical shaker for 48 hours to equilibrate. Samples were centrifuged and aliquots removed for quantification as described previously. This process was repeated for a total of three desorption steps. Recovery of <sup>14</sup>C from check samples was > 97%.

**Mobility.** Methiozolin mobility was characterized using a thin-layer chromatography technique with soil similar to previous studies (Goetz et al. 1986, 1989; Helling and Turner 1968; Helling 1971; Strebe and Talbert 2001). Nine soils, previously described, were deposited on 20 by 20 cm glass plates as a water slurry and then air-dried. Soil layer thickness was approximately 3 mm. Soil was removed from the bottom 2 cm of each plate. Three drops (10 µL each) of experimental spotting solution of methiozolin and isoxaben (described previously) were individually placed 3 cm from the base of each plate resulting in six total spots along the bottom of each plate. Plates were developed in 0.01 M CaCl<sub>2</sub> solution for a distance of ~18 cm, with the exception of USGA > 15, which only developed to ~10 cm. Plates were developed at a 37 degree angle from level. The angle was necessary due to the high sand content in some soils, which would cause the soil to slide down and off the plate when greater angles were used. Additionally, a sponge was affixed to the bottom of each plate to prevent soil slumping, similar to Strebe and Talbert (2001).

After developing, plates were oven dried (70 C for 12 hours) with the angle maintained. Plates were analyzed for radioactivity using a Bioscan AR-2000 plate scanner (Eckert & Ziegler, Washington, DC). Distance from the origin to the wetting front was divided into 10 increments, and the amount of radioactivity detected in each portion quantified by the scanner. Distance from the origin to the center of the displaced peak was used to determine the retardation factor ( $R_f$ ) value is similar to previous studies (Grey et al. 1996, 1997). A total of 4 plates per soil were analyzed.

**Data Analysis.** Data were analyzed using SAS (SAS® Institute v. 9.1, Cary, NC). Based on ANOVA, experimental repetition by soil interactions were not significant for sorption, desorption, or mobility studies ( $P > 0.05$ ). Therefore data were pooled across experimental repetition within each study for subsequent analysis. Means of  $K_d$ ,  $K_{oc}$ , percent methiozolin in solution, and  $R_f$  were separated using 95% confidence intervals adjusted for multiple comparisons using PROC GLIMMIX (Littell et al. 2006). Effect of soil pH, clay content, and organic matter content on methiozolin soil sorption used PROC REG to determine significant linear associations. Sorption kinetics and organic matter association with sorption were evaluated using SigmaPlot (Systat Software Inc. SigmaPlot v. 11.0, San Jose, CA) with the nonlinear plateau model:

$$\text{Sorption} = Y_0 + a (1 - e^{-K * X}) \quad [4]$$

where sorption represents the percent of methiozolin sorbed to soil from that applied (3 mg methiozolin  $\text{kg}^{-1}$  soil),  $a$  and  $K$  are constants generated from the analysis,  $Y_0$  is the y-intercept,  $X$  is time following exposure (in hours) for sorption kinetic experiments and  $X$  is % organic matter for organic matter association with sorption experiments, respectively. A lognormal distribution

was necessary to satisfy model assumptions for  $R_f$  data analysis; data were back transformed for presentation.  $R_f$  analysis was conducted separately for methiozolin and isoxaben since herbicide by soil was a significant interaction ( $P < 0.001$ ). Pearson's correlation coefficients were calculated between  $K_d$  and  $R_f$  values using PROC CORR.

## Results and Discussion

### Sorption.

*Kinetics.* Kinetics experiments revealed that maximum methiozolin sorption was  $> 95\%$  of that applied at 24 hours following exposure (Figure 3). Maximum predicted sorption was 2.0 and 1.9  $\text{mg kg}^{-1}$  for newly constructed USGA sand and field soil, respectively. The kinetics of methiozolin sorption is typical of most herbicides, where an initial fast sorption to the most accessible sites is followed by a slower sorption to less accessible sites (Oliveira et al. 2011; Wauchope et al. 2002). While a majority of methiozolin sorption occurred by 24 hours after exposure, there is evidence that sorption of hydrophobic organic chemicals such as methiozolin to organic matter is retarded by intraorganic matter diffusion (Brusseau et al. 1991). Since other soils evaluated had greater amounts of organic matter than those used in these kinetics studies (Table 7), a 48 hours equilibration period was used in sorption studies.

*Sorption.* Sorption coefficients ranged from 0.4  $\text{mL g}^{-1}$  for sand to 29.4  $\text{mL g}^{-1}$  for sand with 20% peat (Table 8). Field soil had a  $K_d$  value of 6.4  $\text{mL g}^{-1}$ . Across all soil media, average  $K_d$  was 13.8  $\text{mL g}^{-1}$ . Soil media with 0.3 to 6.1% organic matter resulted in 9.5 to 32% of applied methiozolin in solution after equilibrium. Across all soil media, 28% of methiozolin remained in soil solution after equilibrium, and therefore it is immediately available for plant root uptake.

Methiozolin soil sorption was dependent on soil physical characteristics. Sorption coefficients and organic matter content were linearly associated ( $P < 0.001$ ;  $R^2 = 0.66$ ); the relationship of best fit was  $K_d = 7.692 + 1.1191(\% \text{ organic matter})$ . A subset of the data was used (sand with 0, 2.5, 5.0, 10 and 20% peat) to further analyze the influence of organic matter content. From this analysis, percent methiozolin sorption to soil sharply increased between 0 and 3.8% organic matter (Figure 4). Organic matter content  $\geq 3.8\%$  resulted in  $\geq 70\%$  sorption, and organic matter content  $\geq 6.1\%$  resulted in  $\geq 85\%$  sorption. This relationship is consistent with other studies, which indicate that as organic matter increases, herbicide sorption increases (Harris 1966; Harris and Sheets 1965; Nearpass 1965; Obrigawitch et al. 1981; Stougaard et al. 1990; Talbert and Fletchall 1965; Upchurch and Mason 1962; Weber et al. 1969). Soil sorption coefficients were not found to be linearly associated with clay content. The molecular structure of methiozolin does not possess a cationic site and therefore is not likely to be retained by clay particles. However, clay content in the soils used in this study was both low and narrow in range (1.25 to 7.5%). While Shea (1989) concluded that pesticides have a greater attraction to organic matter compared to clay particles by providing more interaction sites, future research is needed to further evaluate methiozolin sorption to soil in relation to clay content.

The  $K_d$  values observed are consistent with other soil-active herbicides (Table 9). These data corroborate previous researchers who reported that methiozolin has soil activity for control of annual bluegrass (Brosnan et al. 2013a; Flessner et al. 2013; Koo et al. 2014; and McCullough et al. 2013).

Herbicides with high soil activity may also be prone to leaching. Notably, methiozolin has  $K_d$  and  $K_{oc}$  values greater than herbicides that have known potential for groundwater contamination (e.g., tebuthiuron), with the exception of the  $K_d$  value of sand with 0.1% organic

matter, which is  $0.4 \text{ mL g}^{-1}$  (Senseman 2007; Wauchope et al. 1992) (Table 9). Methiozolin  $K_d$  values to soils with  $\geq 0.3\%$  organic matter were  $> 6.4 \text{ mL g}^{-1}$ . *S*-metolachlor possesses similar  $K_d$  and  $K_{oc}$  values to methiozolin (Table 9). *S*-metolachlor was found to have insignificant leaching when soil organic matter was  $> 2\%$ , but leaching may occur in soils with  $< 1\%$  organic matter (Obrigawitch et al. 1981). It is unlikely that methiozolin will result in groundwater contamination when applied appropriately to soils with  $> 0.3\%$  organic matter but leaching may occur in soils with less organic matter.

*Effect of pH.* Soil pH had negligible influence on methiozolin sorption to the two soils evaluated (Table 10). Linear association of soil pH and  $K_d$  in newly constructed USGA sand was not significant ( $P = 0.910$ ); linear association in the field soil was marginally significant ( $P = 0.050$ ) but had a poor  $R^2$  value (0.09) and was defined as  $K_d = 5.024 + 0.506(\text{pH})$ . Therefore, as soil pH increased from 4.72 to 7.22 in the field soil, the predicted  $K_d$  value increase was only  $1.3 \text{ mL g}^{-1}$ , which is negligible in an agronomic sense. Because methiozolin does not possess an ionizable functional group, this result is not surprising. The lack of effect of soil pH on herbicide sorption to soil has been observed with other herbicides without ionizable functional groups (Farmer and Aochi 1974).

**Desorption by Dilution.** Methiozolin desorption did not occur from the soils by dilution, as indicated by increasing or similar percent sorption as desorption steps progressed (Table 11). Methiozolin sorption significantly increased from initial sorption to the third desorption step in all soils except sand, newly constructed USGA sand, and USGA >15. In a review by Wauchope et al. (2002), excellent explanations for this effect are described (section 2.4). Possible reasons include abrasive wear exposing new surface area or organic matter acting as a swelling polymer

(Wauchope et al. 2002). Regardless, methiozolin does not readily desorb via dilution once sorbed. Similar observations have been made with flumeturon, 2,4,5-T, alachlor, and aminocyclopyrachlor, among others (Koskinen and Cheng 1983; Oliveira et al. 2011; Savage and Wauchope 1974; Wauchope et al. 2002; Xue and Selim 1995). Since desorption was not observed, the methiozolin fraction that sorbs to soil will likely be permanently unavailable for root uptake.

**Mobility.** A herbicide by soil interaction was detected ( $P < 0.001$ ). All subsequent analysis was conducted separately by herbicide.

Methiozolin had very limited soil mobility. The  $R_f$  values were  $< 0.05$  for all soils, except sand where methiozolin had intermediate mobility ( $R_f = 0.46$ ) (Table 12). Methiozolin was significantly more mobile in sand relative to other soils evaluated; the  $R_f$  value in sand (0.464) was approximately 10 times greater than the next highest  $R_f$  value (0.05) in USGA>15. Averaged across all soils evaluated, methiozolin had an  $R_f$  value of 0.07.

Soil mobility of methiozolin was consistent with its chemical properties. Methiozolin has relatively low water solubility ( $3.4 \text{ mg L}^{-1}$ ; Koo et al. 2010) and a hydrophobic  $\log K_{ow}$  value (3.9; Anonymous 2012b), which are indicative of limited soil mobility (Ross and Lembi 1999; Sakaliene et al. 2007).

Isoxaben was also found to have limited soil mobility. The highest  $R_f$  value observed was 0.10 in USGA>15, which was similar to sand with an  $R_f$  value of 0.06 (Table 12). All other soils resulted in  $R_f$  values  $\leq 0.03$ . Averaged across all soils evaluated, isoxaben had an  $R_f$  value of 0.04.



Isoxaben has low water solubility ( $1 \text{ mg L}^{-1}$ ) and is hydrophobic ( $\log K_{ow} = 2.6$ ), which is consistent with limited soil mobility (Ross and Lembi 1999; Sakaliene et al. 2007; Senseman 2007). Jamet and Thoisy-Dur (1988) also evaluated isoxaben using soil thin-layer chromatography in several French soils and reported the herbicide to have an  $R_f$  value of 0.0. Therefore, these results are consistent with previous research.

Herbicide soil mobility is often estimated using soil thin-layer chromatography techniques. Helling and Turner (1968) proposed a herbicide soil mobility classification system based on the soil mobility of 16 pesticides in Hagerstown silty clay loam and further developed by Helling (1971) to include 40 pesticides. This classification system is from 1 to 5 with 1 being the least soil mobile and 5 being the most soil mobile. Based on this classification system, methiozolin is a class 1 ( $R_f = 0$  to  $< 0.1$ ), or least mobile in soils with  $\geq 0.3\%$  organic matter. In soils with  $\geq 0.3\%$  organic matter, methiozolin mobility is similar to that of paraquat ( $R_f = 0.00$ ) and diquat ( $R_f = 0.06$ ) and less mobile than diuron ( $R_f = 0.24$ ) or siduron ( $R_f = 0.30$ ) (Helling 1971). In sand, methiozolin is a class 3 ( $0.35 < R_f < 0.65$ ), or has intermediate soil mobility, similar to flumeturon ( $R_f = 0.50$ ) and atrazine ( $R_f = 0.47$ ) but less mobile than 2,4-D ( $R_f = 0.69$ ) (Helling 1971). Isoxaben is also a class 1 herbicide with an average  $R_f$  value of 0.039. Jamet and Thoisy-Dur (1988) previously reported isoxaben to be a class 1 herbicide.

Correlation between  $K_d$  and  $R_f$  values for methiozolin was observed; Pearson's correlation coefficient was  $-0.465$  ( $P < 0.001$ ). Previous research has found that soil sorption and soil mobility are generally associated in this manner (Helling 1971; Ladlie et al. 1976a; Sakaliene et al. 2007; Stougaard et al. 1990; Strebe and Talbert 2001). Therefore, this research is consistent with previous reports.

Overall, results indicate that ~24% of applied methiozolin remains available for root uptake, but binds sufficiently to soil so as to resist leaching displacement. Thus, these studies indicate methiozolin is available in soil for herbicidal activity that has been observed in selective placement and field studies (Brosnan et al. 2013a; Flessner et al. 2013; Hwang et al. 2005; Koo et al. 2014; McCullough et al. 2013).

Table 7. Chemical and physical properties of soils used for methiozolin absorption, desorption, and mobility studies.

soil		pH <sup>a</sup>	organic matter <sup>b</sup>	sand <sup>c</sup>	silt <sup>c</sup>	clay <sup>c</sup>
abbreviation	description					
				-----%		
sand	USGA sand <sup>d</sup>	8.1	0.1	97.5	0.6	1.9
sand + 2.5P	USGA sand + 2.5% peat <sup>e</sup>	7.0	3.8	96.9	1.9	1.3
sand + 5.0P	USGA sand + 5.0% peat	6.4	6.1	95.0	2.5	2.5
sand + 10P	USGA sand + 10% peat	6.3	14.4	95.0	2.5	2.5
sand + 20P	USGA sand + 20% peat	6.3	20.1	90.6	7.5	1.9
new USGA	newly constructed USGA putting green root-zone	4.9	0.3	97.5	0.0	2.5
USGA10	10 years as USGA putting green root-zone <sup>f</sup>	4.8	0.8	88.1	9.4	2.5
USGA>15	> 15 years as USGA putting green root-zone <sup>f</sup>	5.2	0.8	96.3	2.5	1.3
field	soil collected from Wickham sandy loam series <sup>g</sup>	6.3	1.7	78.8	13.8	7.5

<sup>a</sup> pH determined by 1:1 soil:deionized water mixture (Soil Survey Staff 2004).

<sup>b</sup> Organic matter content determined via combustion (Elementar, Elementar Americas, Inc.) according to the methods of Yeomans and Bremner (1991) and Baldock and Nelson (2000).

<sup>c</sup> Soil textural analysis was conducted using the hydrometer method (Soil Survey Staff 2004)

<sup>d</sup> Abbreviations: USGA, United States Golf Association.

<sup>e</sup> Sphagnum peat moss combined dry wt wt<sup>-1</sup>.

<sup>f</sup> USGA root-zones collected from the Auburn University Turfgrass Research Unit in Auburn, AL (32.29°N, 85.53°W) that were constructed to meet USGA specifications for root-zone content.

<sup>g</sup> Field soil was collected from the soil surface to 25 cm depth after cultivating from the Wickham sandy loam series at Auburn University's Plant Breeding Unit near Tallassee, AL (32.34°N, 85.29°W).

Table 8. Soil sorption coefficient ( $K_d$ ), organic carbon coefficient ( $K_{oc}$ ), and percent methiozolin in solution values for batch equilibrium sorption experiments with methiozolin at 3 mg kg soil<sup>-1</sup>.

soil <sup>a</sup>	$K_d$			$K_{oc}$			methiozolin in solution after equilibrium		
	mean <sup>b</sup>	95% confidence limits <sup>b</sup>		mean	95% confidence limits		mean	95% confidence limits	
	-----mL g <sup>-1</sup> -----						-----%-----		
sand	0.4F	-1.4	2.3	486.1C	216.7	755.4	88.3A	82.3	94.2
sand + 2.5P	8.9E	7.6	10.3	365.9C	110.4	621.4	30.0B	25.6	34.4
sand + 5.0P	18.1C	16.2	20.0	504.0C	260.4	747.6	14.4C	8.4	20.3
sand + 10P	24.6B	22.4	26.8	290.9C	5.2	576.5	11.2C	4.2	18.1
sand + 20P	29.4A	27.6	31.3	249.2C	16.0	482.5	9.5C	3.8	15.2
new USGA	7.4E	5.6	9.1	3864.3A	3620.7	4107.9	30.0B	24.6	35.5
USGA10	13.5D	11.7	15.3	2917.0B	2673.4	3160.7	18.7BC	13.1	24.4
USGA>15	15.7CD	13.8	17.5	3327.9AB	3084.3	3571.5	16.1C	10.2	22.0
field	6.4E	4.2	8.6	644.0C	358.3	929.6	32.1B	25.2	39.1

<sup>a</sup> Soil abbreviations, descriptions, physical, and chemical properties are presented in Table 7.

<sup>b</sup> Means separated using SAS PROC GLIMMIX with 95% confidence intervals adjusted for multiple comparisons. Means with a shared letter are not significantly different within column.

Table 9. Soil sorption ( $K_d$ ) and soil organic carbon sorption ( $K_{oc}$ ) coefficients of herbicides related to soil activity.<sup>a</sup>

$K_d$ value	proportion		qualitative herbicidal activity in soil	examples:		
	adsorbed to soil	remaining in solution		herbicide	$K_d$	$K_{oc}$
$\text{mL g}^{-1}$	-----%				----- $\text{mL g}^{-1}$ -----	
10,000	>99.9	<0.01	no soil activity	paraquat	no soil activity	1,000,000
1,000	99.9	0.1	minimal soil activity	glyphosate	324 to 600	24,000
100	99	1		prodiamine	20 to 400	13,000
10	91	9	soil active; activity increases as $K_d$ value decreases	methiozolin	0.4 to 29	250 to 3,860
5	83	17		S-metolachlor	1.2	200
1	50	50	very soil active; also leachable	aminopyralid	0.03 to 0.72	10.8
0.5	33	67		tebuthiuron	0.11	22 to 91
0.1	10	90	does not occur in typical soil			

<sup>a</sup> Compiled from Senseman (2007), Wauchope et al. (1992) and current research.

Table 10. Effect of pH on soil sorption coefficients ( $K_d$ ) for field soil and USGA sand based root-zone with methiozolin added at 3 mg kg soil<sup>-1</sup>.

soil <sup>a</sup>	pH	$K_d$ <sup>b</sup>	sorption to soil <sup>b</sup>
		-----mL g <sup>-1</sup> -----	-----%-----
new USGA	5.03	6.4 ± 0.5	70.0 ± 1.99
	6.02	6.7 ± 0.2	73.0 ± 0.87
	6.77	6.2 ± 0.7	73.9 ± 1.14
	7.28	6.6 ± 0.7	73.4 ± 1.07
	mean ---	6.5 ± 0.3	72.5 ± 0.87
field	4.72	7.3 ± 0.5	67.5 ± 1.73
	6.02	8.2 ± 0.4	68.8 ± 0.71
	6.87	8.7 ± 0.6	65.7 ± 2.17
	7.22	8.5 ± 0.5	67.4 ± 2.26
	mean ---	8.1 ± 0.3	67.4 ± 0.88

<sup>a</sup> Soil abbreviations, descriptions, physical, and chemical properties are presented in Table 7.

<sup>b</sup> Mean ± standard error.

Table 11. Comparison of methiozolin soil sorption (%) after initial sorption and subsequent dilution desorption steps in batch equilibrium experiments with 0.1 M CaCl<sub>2</sub>.

soil <sup>a</sup>	sorption			desorption step <sup>b</sup>								
	mean <sup>c</sup>	95% confidence limits <sup>c</sup>		1			2			3		
		mean	lower	upper	mean	lower	upper	mean	lower	upper	mean	lower
	-----%-----											
sand	11.7 A	-4.0	27.4	22.9 A	6.4	39.3	25.7 A	7.3	44.1	7.8 A	-13.5	29.0
sand + 2.5P	70.0 B	64.8	75.1	77.6 AB	71.6	83.5	78.8 AB	72.9	84.7	82.0 A	76.1	87.9
sand + 5.0P	85.6 B	84.6	86.7	85.8 B	84.7	86.9	87.0 B	85.9	88.1	89.3 A	88.2	90.4
sand + 10P	88.8 B	87.6	90.1	89.1 AB	87.8	90.3	89.7 AB	88.4	90.9	91.4 A	90.1	92.6
sand + 20P	90.5 B	89.5	91.6	91.1 B	90.1	92.2	91.0 B	90.0	92.0	93.1 A	92.1	94.2
new USGA	70.0 A	67.1	72.9	70.8 A	67.6	73.9	70.9 A	67.7	74.0	72.5 A	69.4	75.7
USGA10	81.3 B	79.0	83.5	83.3 AB	81.0	85.5	85.7 AB	82.9	88.5	87.8 A	85.0	90.6
USGA>15	83.9 A	82.7	85.1	85.0 A	83.7	86.2	84.4 A	83.2	85.6	86.1 A	84.9	87.3
field	67.9 B	63.4	72.3	71.1 B	66.6	75.5	75.6 AB	71.8	79.4	79.0 A	75.2	82.8

<sup>a</sup> Soil abbreviations, descriptions, physical, and chemical properties are presented in Table 7.

<sup>b</sup> In each desorption step, 3 mL of 0.1 M CaCl<sub>2</sub> solution replaced the 3 mL of solution that was removed from the test tube for methiozolin quantification. A 48 hours equilibration period was used for each step. Initial methiozolin concentration was 3 mg kg soil<sup>-1</sup>.

<sup>c</sup> Means separated using SAS PROC GLIMMIX with 95% confidence intervals adjusted for multiple comparisons. Means with a shared letter are not significantly different within row.

Table 12. Methiozolin and isoxaben retardation factors ( $R_f$ ) determined by thin-layer soil chromatography.

soil <sup>a</sup>	methiozolin			isoxaben		
	mean <sup>b</sup>	95% confidence limits <sup>b</sup>		mean <sup>b</sup>	95% confidence limits <sup>b</sup>	
	----- $R_f$ -----					
sand	0.46 A	0.32	0.67	0.06 AB	0.04	0.09
sand + 2.5P	0.02 C	0.01	0.03	0.02 C	0.01	0.03
sand + 5.0P	0.02 BC	0.02	0.03	0.02 C	0.01	0.03
sand + 10P	0.02 BC	0.02	0.03	0.03 BC	0.02	0.05
sand + 20P	0.02 BC	0.02	0.03	0.02 C	0.02	0.03
mew USGA	0.01 C	0.01	0.02	0.03 BC	0.02	0.04
USGA10	0.02 BC	0.02	0.03	0.03 BC	0.02	0.04
USGA>15	0.05 B	0.03	0.07	0.10 A	0.07	0.15
field	0.02 C	0.01	0.03	0.03 BC	0.02	0.04

<sup>a</sup> Soil abbreviations, descriptions, physical, and chemical properties are presented in Table 7.

<sup>b</sup> Means separated using SAS PROC GLIMMIX with 95% confidence intervals adjusted for multiple comparisons. Means with a shared letter are not significantly different within column.



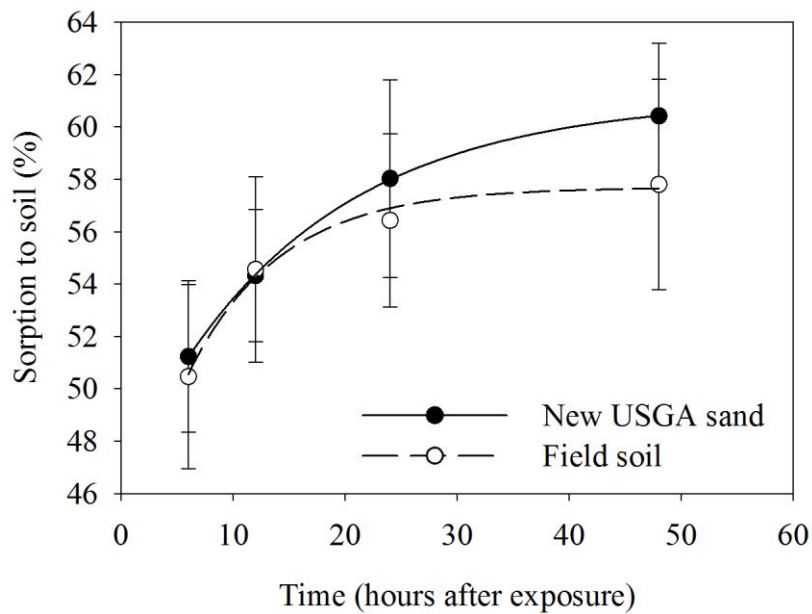


Figure 3. Methiozolin soil sorption from batch equilibrium experiments as a function of time for new USGA sand and field soil (see Table 7 for soil chemical and physical parameters) with nonlinear regression analysis (Equation 4). New USGA sand was described by  $\text{Sorption} = 46.56 + 14.54(1 - e^{-0.064 \cdot \text{hours}})$ . Field soil was described by  $\text{Sorption} = 42.86 + 14.83(1 - e^{-0.122 \cdot \text{hours}})$ . Points are means surrounded by standard error.

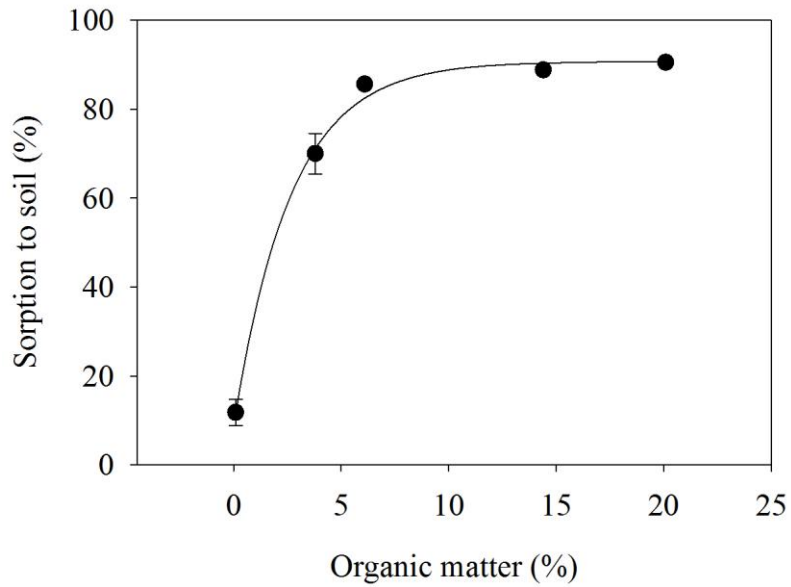


Figure 4. Relationship between soil sorption (%) and organic matter content (%) for USGA sand with 0, 2.5, 5.0, 10, or 20% (w/w) peat in batch equilibrium sorption experiments (see Table 7 for soil chemical and physical parameters). Nonlinear regression analysis resulted in  $\text{Sorption} = 8.50 + 82.20(1 - e^{-0.377 \cdot \text{organic matter (\%)}})$ . Points are means surrounded by standard error.

## **Annual Bluegrass (*Poa annua*) Control with Methiozolin Tank-Mixed with Nutrients**

### **Introduction**

Annual bluegrass (*Poa annua* L.) is a problematic weed in golf course putting greens due to its disruption of utility and aesthetics and its ability to survive and thrive at low mowing heights (Beard 1973; Mitch 1998). Annual bluegrass is perhaps the most troublesome weed in putting greens, in part due to few control options. There are no selective post-emergent (POST) herbicides for annual bluegrass control in creeping bentgrass (*Agrostis stolonifera* L.) putting greens currently registered in the United States (Brosnan et al. 2013a). Pre-emergent (PRE) herbicide options are limited to bensulide and fenarimol. However, PRE control is complicated by the long germination period of annual bluegrass and perennial biotypes (Beard et al. 1978; Callahan and McDonald 1992; Itoh et al. 1997; Kaminski and Dernoeden 2007; McElroy et al. 2004). The growth regulators paclobutrazol and flurprimidol have resulted in suppression of annual bluegrass and are registered for use in putting greens (Johnson and Murphy 1995; Johnson and Murphy 1996; Woosley et al. 2003). Other agrochemicals, such as amicarbazone and ethofumesate, have been researched for use in creeping bentgrass, but are not registered for putting green use and have resulted in inconsistent control and intolerable turfgrass injury in some cases (Kohler and Branham 2002; McCullough et al. 2010).

Methiozolin (5-(2,6-difluoro-benzyloxymethyl)-5-methyl-3-(3-methyl-thiophen-2-yl)-4,5-dihydro-isoxazole) is a herbicide that selectively controls annual bluegrass in creeping bentgrass and hybrid bermudagrass (*Cynodon dactylon* × *C. transvaalensis* Burt-Davy)

(Brosnan et al. 2013a; Hwang and Koo 2009; Koo et al. 2014; McCullough and Gómez de Barreda 2012; McCullough et al. 2013). Methiozolin is currently registered for this use in South Korea and Japan, and registration is currently being pursued in the United States. Methiozolin has both PRE and POST herbicidal activity on annual grass weeds (Brosnan et al. 2013a; McCullough et al. 2013). PRE control is limited by relatively quick aerobic soil microbe metabolism; methiozolin has a reported half-life of 49 days under such conditions (Hwang et al. 2013). Methiozolin is absorbed by the roots and foliage of annual bluegrass, but root exposure is required for efficacious annual bluegrass control (Brosnan et al. 2013a; Koo et al. 2014; McCullough et al. 2013). Selectivity between annual bluegrass and creeping bentgrass have been attributed to differential absorption and translocation (McCullough et al. 2013; Yu and McCullough 2014).

Annual bluegrass control with methiozolin requires multiple, sequential applications that total 2.0 to 3.36 kg ai ha<sup>-1</sup> (Brosnan et al. 2013a; McCullough et al. 2013). Sequential applications are more efficacious than single applications (Brosnan et al. 2013a). Fall application has been reported to be more efficacious than spring applications (Koo et al. 2014). Greater fall control may be due to greater methiozolin foliar and root absorption and greater translocation from root tissues at higher temperatures (McCullough et al. 2013).

Golf course managers frequently tank-mix herbicides with fertilizers to reduce time and labor expenditures (Fearis 2002). Tank-mixing fertilizers with herbicides can influence the efficacy of the herbicide application or have no effect. Chelated iron was found to have no influence on bispyribac-sodium efficacy on annual bluegrass (McCullough and Hart 2009). Similarly, iron and nitrogen tank-mixed with bispyribac-sodium did not reduce annual bluegrass control efficacy, but effectively masked discoloration of creeping bentgrass relative to

bispyribac-sodium alone (McDonald et al. 2006). Iron tank-mixed with ethofumesate plus fluprimsol resulted in transient improved quality of creeping bentgrass and reduced discoloration (Johnson and Carrow 1995). Iron sulfate tank-mixed with MSMA resulted in reduced southern crabgrass (*Digitaria sanguinalis* (L.) Scop.) control and bermudagrass (*Cynodon dactylon* (L.) Pers.) injury relative to MSMA alone (Massey et al. 2006). Ammonium sulfate is commonly added to spray solutions to reduce the antagonistic effects of hard water on weak acid herbicides including 2,4-D and glyphosate by reducing interactions in spray solution with metal cations present in hard water such as calcium (O'Sullivan et al. 1981; Roskamp et al. 2013). Ammonium sulfate increased the speed of sethoxydim absorption in large crabgrass (*Digitaria sanguinalis* (L.) Scop.) when tank-mixed (Jordan et al. 1989) and increased glufosinate efficacy, absorption, and translocation in several weed species (Maschhoff et al. 2000).

Assessing methiozolin efficacy for annual bluegrass control when tank-mixed with common fertilizers has not been done. The objectives of this research were to evaluate methiozolin for annual bluegrass control and creeping bentgrass safety when tank-mixed with ammonium sulfate and iron sulfate.

## **Materials and Methods**

**General information.** Field research was conducted at the Auburn University Turfgrass Research and Education Center in Auburn, AL (32.34°N, 85.29°W) on a 'Crenshaw' creeping bentgrass putting green with an endemic annual bluegrass population. Soil type was a Marvyn Sandy Loam (fine-loamy, kaolinitic, thermic Typic Kanhapludult) with a pH of 6 and 1.1% organic matter. Fertility, irrigation, disease control, and pest control practices were conducted

according to standard practices. The turfgrass was cut three times per week at 0.32 cm. Three separate experiments were conducted according to experimental objectives and each was repeated in time. All experiments were conducted as a randomized complete block design with a minimum of 3 replications. All treatments were applied at 280 L ha<sup>-1</sup> with a handheld four nozzle boom (TeeJet TP8002VS nozzles on 25 cm spacing; Spraying Systems Company, Wheaton, IL) to 1.5 m by 1.5 m plots. Data collected varied by experiment, but all experiments visually assessed annual bluegrass control relative to the non-treated check on a 0 (no control) to 100 (complete plant death) scale. Creeping bentgrass injury was evaluated using a similar 0 to 100 scale, with a score of 20 representing the maximum level of creeping bentgrass injury considered commercially acceptable, similar to previous researchers (Johnson and Murphy 1995). Statistical analysis was conducted using SAS PROC GLM (SAS® Institute v. 9.1, Cary, NC). Effects were considered significant when P < 0.05. When subsequent regression analysis was necessary, SigmaPlot software was used (SigmaPlot® 11, Systat Software Inc. San Jose, CA) using linear or, in some cases, the nonlinear plateau models:

$$\text{Annual bluegrass control (\%)} = Y_0 + a (1 - e^{-b \cdot \text{rate}}) \quad [1]$$

$$\text{Creeping bentgrass injury (\%)} = Y_0 + a \cdot e^{-b \cdot \text{rate}} \quad [2]$$

where Y<sub>0</sub> is the y-intercept, a and b are constants, and rate is methiozolin rate in kg ai ha<sup>-1</sup>.

**Methiozolin Tank-Mixed with Ammonium Sulfate.** Methiozolin (MRC-01; Moghu Research Center, Daejeon, Korea) was applied at 0.84, 1.68, and 3.36 kg ai ha<sup>-1</sup> with and without ammonium sulfate at 12.2 kg N ha<sup>-1</sup> to determine the effects of methiozolin rate and ammonium sulfate on annual bluegrass control and creeping bentgrass safety. An ammonium sulfate alone treatment at 12.2 kg N ha<sup>-1</sup> was also included as well as a nontreated check. All treatments were

applied three times sequentially with the second application two weeks after the initial application and the third application 4 weeks after the second application. Application dates were 18 January, 31 January, and 1 March 2012 for experimental run one and 15 January, 1 February, and 27 February 2014 for experimental run two. Annual bluegrass control and creeping bentgrass injury data were collected 2, 5, 8, and 10 weeks after initial treatment (WAIT).

**Methiozolin Tank-Mixed with Iron Sulfate.** Methiozolin was applied at 0.28 and 0.56 kg ai ha<sup>-1</sup> with and without iron sulfate (Ferromec; PBI/Gordon Corporation, Kansas City, MO) at 0.96 kg Fe ha<sup>-1</sup> to determine the effects of methiozolin rate and iron sulfate on annual bluegrass control and creeping bentgrass safety. Paclobutrazol (Trimmit® 2SC; Syngenta Crop Protection, LLC, Greensboro, NC) was applied at 0.28 kg ai ha<sup>-1</sup> with and without iron sulfate at 0.96 kg Fe ha<sup>-1</sup> as a comparison treatment as well as iron sulfate alone at 0.96 kg Fe ha<sup>-1</sup>. A nontreated check was also included. All treatments were applied twice sequentially, 24 days apart. Application dates were 8 November and 2 December 2010 for the first experimental run and 14 January and 6 February 2014 for the second experimental run. Annual bluegrass control and creeping bentgrass injury data were collected 1, 2, 4, 8, 12, and 16 WAIT.

**Methiozolin, Amicarbazone, and Ethofumesate Alone and Tank-Mixed with Iron and Nitrogen.** Methiozolin, amicarbazone, and ethofumesate applied with and without iron sulfate and ammonium sulfate, respectively, were compared for annual bluegrass control, creeping bentgrass safety, and effects on turfgrass quality and normalized difference vegetative index (NDVI). Treatments included methiozolin at 0.84 kg ai ha<sup>-1</sup>, amicarbazone (Xonerate®; Arysta LifeScience North America, LLC, Cary, NC) at 0.07 kg ai ha<sup>-1</sup>, and ethofumesate (Prograss®;

Bayer Environmental Science, Research Triangle Park, NC) at  $1.13 \text{ kg ai ha}^{-1}$ . Each of these agrochemicals were applied alone, with iron sulfate (15% Iron Sulfate; Bonide Products Inc., Oriskany, NY) at  $0.96 \text{ kg Fe ha}^{-1}$ , and with ammonium sulfate at  $12.2 \text{ kg N ha}^{-1}$ . Additionally, iron sulfate and ammonium sulfate were applied alone. A nontreated check was also included. All treatments were applied four times sequentially every 2 weeks, with application dates of 1 November, 14 November, 30 November, and 13 December 2011 for experimental run one and 15 January, 1 February, 14 February, and 27 February 2014 for experimental run two. Visual annual bluegrass control and turfgrass quality data were collected 1, 2, 3, 4, 6, 8, 10, and 14 WAIT. Turfgrass quality was rated on a 1 to 9 scale with 1 corresponding to inferior quality, 7 to commercially acceptable quality, and 9 to ideal quality. NDVI evaluation was also conducted 4, 6, and 10 WAIT using a Field Scout™ TCM 500 Turf Color Meter (Spectrum™ Technologies, Inc. Plainfield, IL). This device measures red ( $660 \pm 5 \text{ nm}$ ; Red) and near-infrared ( $850 \pm 5 \text{ nm}$ ; NIR) light reflectance from an internal light source and calculates NDVI as  $(\text{NIR} - \text{Red}) / (\text{NIR} + \text{Red})$ . Three subsamples were collected per plot, and their mean was used in analysis. Pearson's correlation coefficients were calculated between Turfgrass quality and NDVI response values using PROC CORR in SAS.

## Results and Discussion

**Methiozolin Tank-Mixed with Ammonium Sulfate.** Annual bluegrass symptoms of methiozolin treatment were characterized by mild chlorosis and growth reduction leading to eventual death. Symptoms were not evident until 5 WAIT, the first rating date where control was observed. For this reason, data analysis and presentation excluded data prior to 5 WAIT. Data were pooled across experimental runs as a significant run by treatment interaction was not



detected. Similarly, a significant ammonium sulfate by methiozolin rate interaction and an ammonium sulfate main effect were not detected, indicating that the addition of ammonium sulfate did not influence annual bluegrass control with methiozolin. Time after treatment was a significant effect; as time after treatment increased, control increased at all methiozolin rates (Figure 5). Also, nonlinear regression analysis indicated that as methiozolin rate increased annual bluegrass control also increased. Control was greatest 10 WAIT and was 78, 99, and 100% from methiozolin applied three times sequentially at 0.84, 1.68, and 3.36 kg ai ha<sup>-1</sup>, respectively. Previous research indicates that annual bluegrass is well controlled from multiple, sequential applications totaling 2.0 to 3.36 kg ai ha<sup>-1</sup> of methiozolin (Brosnan et al. 2013a; McCullough et al. 2013). This research applied a total of 2.52, 5.04, and 10.08 kg ai ha<sup>-1</sup> of methiozolin, which resulted in 78, 99, and 100% control, respectively, at the final rating 10 WAIT. Therefore, this research indicates that greater methiozolin rates may be required to obtain commercially acceptable control (> 90%). Regression analysis predicts that 7.32 kg ai ha<sup>-1</sup> of methiozolin is necessary to obtain 90% control, 10 WAIT (following the application regime for this research). This difference is likely due to cooler temperatures and timing of application (research was initiated in January) compared to previous research, which indicates that fall applications are more efficacious than spring applications (Koo et al. 2014) likely due to greater methiozolin translocation from root tissues at higher temperatures; however, annual bluegrass injury was similar across temperatures from 10 to 30 C (McCullough et al. 2013).

Creeping bentgrass injury was observed and was characterized by growth reduction followed by chlorosis, followed by necrosis. Mild injury (< 20%) was observed 5 WAIT, but most injury was observed 8 and 10 WAIT (Figure 6). For this reason, only data from 8 and 10 WAIT are presented. Data were pooled across experimental runs as a significant run by

treatment interaction was not detected. Time after treatment was a significant effect; generally, as time after treatment increased creeping bentgrass injury also increased. However, recovery from injury was observed from methiozolin applied sequentially at 0.84 kg ai ha<sup>-1</sup> without ammonium sulfate and 1.68 kg ai ha<sup>-1</sup> with and without ammonium sulfate. Initial injury was 4 to 10% for these treatments (5 WAIT) and recovered to ≤ 3% 10 WAIT. A significant methiozolin rate by ammonium sulfate interaction was detected 8 and 10 WAIT, which can also be observed in the nonlinear regression analysis (Figure 6). The addition of ammonium sulfate significantly reduced creeping bentgrass injury from methiozolin applied sequentially at 3.36 kg ai ha<sup>-1</sup>; injury was reduced from 26 to 8% 8 WAIT and 38 to 17% 10 WAIT. Methiozolin has been reported to be noninjurious to creeping bentgrass (Koo et al. 2014). However, some creeping bentgrass injury has been reported. Brosnan et al. (2013a) reported < 2% injury from all treatments, which had a maximum of 3.0 kg ai ha<sup>-1</sup> applied. Other research indicates that 2.0 kg ai ha<sup>-1</sup> resulted in 10% injury 4 weeks after treatment (Brosnan et al. 2013b). Research also indicates that reseeding of creeping bentgrass should be delayed 2 weeks after methiozolin application at ≤ 1.12 kg ai ha<sup>-1</sup> and 6 weeks after methiozolin application at 2.24 g ai ha<sup>-1</sup> due to reductions in new turfgrass cover (McCullough and Gómez de Barreda 2012). Yu and McCullough (2014) reported that methiozolin > 6.72 kg ai ha<sup>-1</sup> would be necessary to result in 50% creeping bentgrass injury. Therefore, this research agrees with previous research which indicates that methiozolin applied at 1.0 kg ai ha<sup>-1</sup> is very safe to creeping bentgrass, but rates greater than 2.0 kg ai ha<sup>-1</sup> can result in creeping bentgrass injury. The January initial application timing likely exacerbated creeping bentgrass injury relative to warmer application timings. Previous research indicates that injury was two- and four-fold greater at 10 C compared to 20 and 30 C, respectively, in growth chamber experiments (McCullough et al. 2013).

Ammonium sulfate has been shown to increase herbicidal activity of weak acid herbicides when mixed in hard water (O'Sullivan et al. 1981; Roskamp et al. 2013). However, methiozolin is not a weak acid, which accounts for the lack of this observation in this research. Ammonium sulfate has also resulted in increased speed of absorption and translocation when tank-mixed (Jordan et al. 1989; Maschhoff et al. 2000) but this effect was not tested in this research. Overall, research indicates that methiozolin can be tank-mixed with ammonium sulfate without reductions in annual bluegrass control while reducing injury to creeping bentgrass in some cases.

**Methiozolin Tank-Mixed with Iron Sulfate.** Annual bluegrass symptoms of methiozolin treatment were similar to those previously described. Annual bluegrass control analysis was limited to 12 and 16 WAIT. A significant experimental run by treatment interaction was detected, so data are presented separately. Iron sulfate and time after treatment were also detected to have significant interactions with treatment, so data were analyzed and presented accordingly (Table 13). Control increased as methiozolin rate increased without iron sulfate and was 100 and 83% from methiozolin applied twice at  $1.12 \text{ kg ai ha}^{-1}$  at the final ratings in 2010-11 and 2014, respectively (Figure 7). Contrasts between methiozolin with or without iron sulfate were significant in 2010-11 and indicated that control significantly increased when iron sulfate was added to methiozolin at  $0.28$  and  $0.56 \text{ kg ai ha}^{-1}$  (Table 13). These same contrasts were not significant in 2014. Therefore the influence of iron sulfate on control with methiozolin was variable. Paclobutrazol resulted in highly variable control between experimental runs. Control was  $\geq 93\%$  with or without iron sulfate in 2010-11 but  $\leq 67\%$  in 2014. Contrasts between paclobutrazol with or without iron sulfate were not significant in either experimental run,

indicating that iron sulfate did not influence control with paclobutrazol. Paclobutrazol resulted in similar annual bluegrass control as methiozolin at 1.12 kg ai ha<sup>-1</sup> and methiozolin at 0.56 kg ai ha<sup>-1</sup> with iron sulfate in 2010-11 but less control in 2014, 16 WAIT. Therefore annual bluegrass control with paclobutrazol was similar or less than with methiozolin at appropriate rates.

Previous research indicates that annual bluegrass is well controlled from multiple, sequential applications totaling 2.0 to 3.36 kg ai ha<sup>-1</sup> (Brosnan et al. 2013a; McCullough et al. 2013). Total methiozolin alone applications rates were 0.56, 1.12, and 2.24 kg ai ha<sup>-1</sup> in this study, which resulted in 35 to 47%, 53 to 65%, and 83 to 100% control, respectively (Table 13). This research corroborates previous research in creeping bentgrass fairways indicating that 85% or more annual bluegrass control can be obtained; however, six monthly applications at 0.28 kg ai ha<sup>-1</sup> were necessary to achieve this result (Woosley et al. 2003). This research also found differences in control between experimental runs, similar to current research. Control in putting greens is more difficult. Research on creeping bentgrass putting greens found that three sequential applications of paclobutrazol at 0.28 kg ai ha<sup>-1</sup> on 14 day intervals resulted in 29 and 46% control 8 and 12 WAIT, respectively (Jefferies et al. 2013). Johnson and Murphy (1996) reported ≤ 57% control 12 WAIT. Therefore, research conducted in 2010-11 resulted in abnormally high control with paclobutrazol relative to previous research, but research conducted 2014 resulted in similar control.

Differences detected between experimental repetitions are likely due to the difference in initiation timing. The 2010-11 experiment was initiated 8 November, while the 2014 experiment was initiated 14 January. Methiozolin has been reported to be more efficacious for annual bluegrass control when applied in the fall versus spring and at higher temperatures (Brosnan et al. 2013a; McCullough et al. 2013). Additionally, annual bluegrass was at 1 to 2 leaf stage for

the first application in 2010-11 whereas 50% were 3 tiller and 35% were seeding for the first application in 2014. Larger, more mature plants resulted in less control relative to smaller, less mature plants (Chapter 2). This same effect is likely true for paclobutrazol, although research is lacking in this regard.

A significant treatment by experimental run interaction was not detected for creeping bentgrass injury, so data were pooled for analysis and presentation. Creeping bentgrass injury was  $\leq 14\%$  from any treatment at any time during this study (Table 14). No injury was detected until 4 WAIT, so earlier data is not presented. Creeping bentgrass injury symptoms were characterized by growth reduction and mild chlorosis for methiozolin and growth reduction with off-coloring for paclobutrazol. Contrast statements investigating iron sulfate inclusion were not significant, indicating that iron sulfate did not influence creeping bentgrass response to methiozolin or paclobutrazol.

Previous research reports that creeping bentgrass responds with no to mild injury to methiozolin treatment as previously described. Paclobutrazol has been previously reported to be non-injurious (Jefferies et al. 2013) to mildly injurious to creeping bentgrass, characterized by growth reductions (Woosley et al. 2003). Johnson and Murphy (1995, 1996) reported  $< 20\%$  injury following spring applications but approximately 30% injury following fall applications. Therefore this research agrees with previous research.

There is no previous research examining the effects of paclobutrazol tank-mixed with iron sulfate for annual bluegrass control or creeping bentgrass injury. However, Han (2012) examined the effects of the plant growth regulators trinexapac-ethyl and flurimidorol with iron sulfate for annual bluegrass cover and found that there was not a significant interaction, which agrees with this research. Creeping bentgrass response was characterized by turfgrass quality and

color evaluations, which were mostly similar but in some instances improved when iron sulfate was added.

Other research reported that chelated iron was found to have no influence on bispyribac-sodium efficacy on annual bluegrass (McCullough and Hart 2009). Similarly, iron and nitrogen tank-mixed with bispyribac-sodium did not reduce annual bluegrass control efficacy, but effective masked discoloration of creeping bentgrass (McDonald et al. 2006). Iron tank-mixed with ethofumesate plus fluprimidol resulted in transient improved quality of creeping bentgrass and reduced discoloration (Johnson and Carrow 1995).

### **Methiozolin, Amicarbazone, and Ethofumesate Alone and Tank-Mixed with Iron and Nitrogen.**

*Annual bluegrass control.* A significant treatment by experimental run interaction was detected for annual bluegrass control, turfgrass quality, and NDVI data types; therefore data were analyzed and presented by experimental run.

Annual bluegrass control increased as time after treatment increased (Table 15). Control did not exceed 63% until 10 WAIT in 2011-12. Control was greatest ( $\geq 84\%$ ) from treatments including methiozolin and ethofumesate at the final rating 14 WAIT. Treatments including amicarbazone resulted in 60 to 70% control in 2011-12. In 2014, control exceeded 65% 6 WAIT for ethofumesate alone and for many treatments by 8 WAIT, which was quicker than in 2011-12. Similar to 2011-12, control was greatest from treatments including methiozolin and ethofumesate, followed by treatments including amicarbazone. Treatments including ethofumesate resulted in  $\geq 85\%$  control at the final rating 14 WAIT, while treatments including methiozolin resulted in  $\geq 92\%$  control. Amicarbazone resulted in  $< 50\%$  control throughout the

2014 experimental run, which was also much less than the 60 to 70% control that resulted in 2011-12.

Generally, the addition of ammonium sulfate or iron sulfate did not influence control in either experimental run, although some differences were detected (Table 15). Notably, methiozolin with ammonium sulfate resulted in 100% control in 2011-12 but only 60% control in 2014, 14 WAIT. Previous research investigating the addition of iron sulfate or ammonium sulfate has been discussed previously.

Differences detected between experimental repetitions are likely due to the difference in initiation timing. The 2011-12 experiment was initiated November 1 while the 2014 experiment was initiated January 15. Previous research indicates that season of application and other variables can influence annual bluegrass control with these agrochemicals. Methiozolin has been reported to be more efficacious for annual bluegrass control when applied in the fall versus spring and at higher temperatures (Brosnan et al. 2013a; McCullough et al. 2013). Amicarbazone efficacy is also related to temperature, season, and even location (McCullough 2010); annual bluegrass control varied from 23 to 85% control in Indiana following two sequential applications at 0.1, 0.2, and 0.3 kg ai ha<sup>-1</sup>, while the same treatments resulted in 88 to 100% control in New Jersey. These same treatments and locations varied from 23 to 74% control in Indiana to 66 to 74% control when applied in spring. Previous research with ethofumesate in creeping bentgrass fairways indicates that annual bluegrass cover was reduced from fall applications but not spring applications (Woosley et al. 2003). Other research reported > 95% annual bluegrass control in overseeded bermudagrass fairways in 2 of 3 years when ethofumesate was applied at 2.2 kg ha<sup>-1</sup> (Dickens 1979). Coats and Krans (1986) reported ≥ 75% annual bluegrass control following two applications of ethofumesate at 1.1 kg ha<sup>-1</sup> applied in December followed by January. Therefore,

differences between experimental runs due to seasonal effects observed in the current research have been previously reported.

Differences between experimental runs may also be attributed to differences in annual bluegrass growth stage, which have been reported to influence control with methiozolin. Larger, more mature plants resulted in less control relative to smaller, less mature plants (Chapter 2). Approximately 75% of the annual bluegrass population was 2 to 3 tillers in size with 5% seeding for applications in 2011-12, whereas approximately 60% was > 3 tillers in size and 25% seeding at the initial application and 90% seeding at subsequent applications in 2014.

Overall, annual bluegrass control in this study was similar to previous research, was greatest with methiozolin and ethofumesate, and was mostly unaffected by addition of iron sulfate or ammonium sulfate.

*Turfgrass quality.* Turfgrass quality was similar to the nontreated by methiozolin or amicarbazone, regardless of tank-mix partners, with few exceptions (Table 16). Turfgrass quality was reduced relative to the nontreated by ethofumesate, regardless of tank-mix partners, with few exceptions. Reductions in turfgrass quality by ethofumesate were characterized by chlorosis, severe growth reductions, density reductions, and annual bluegrass control, which left gaps in the turfgrass cover. Ammonium sulfate alone resulted in similar turfgrass quality relative to the nontreated in 2011-12, but improved turfgrass quality relative to the nontreated in 2014 at 6 and 10 WAIT. Iron sulfate alone resulted in similar turfgrass quality relative to the nontreated in 2011-12 and 2014 at all rating dates.

Methiozolin tank-mixed with ammonium sulfate resulted in similar or improved turfgrass quality relative to methiozolin alone but improved quality relative to methiozolin tank-mixed with iron sulfate on some occasions (Table 16). Amicarbazone tank-mixed with ammonium



sulfate or iron sulfate resulted in similar or improved turfgrass quality relative to amicarbazone alone. Amicarbazone tank-mixed with ammonium sulfate compared to iron sulfate were similar on all occasions. Tank-mixes with ethofumesate resulted in similar or reduced turfgrass quality relative to ethofumesate alone. Overall, methiozolin and amicarbazone resulted in similar turfgrass quality relative to the nontreated while ethofumesate resulted in unacceptable reductions.

Differences between experimental runs are likely due to different initiation timings as previously mentioned. Previous research indicates that creeping bentgrass injury from methiozolin was two- and four-fold greater at 10 C compared to 20 and 30 C, respectively, in growth chamber experiments (McCullough et al. 2013). Creeping bentgrass injury from amicarbazone has been reported to be greater when applied in fall versus spring (McCullough et al. 2010). Woosley et al. (2003) reported variation in creeping bentgrass growth reduction to ethofumesate between experimental runs.

Previous research indicates that ethofumesate can maintain or improve fairway turfgrass quality following multiple monthly applications, which is attributed to annual bluegrass seedhead suppression (Woosley et al. 2003). This research clearly indicates that when cut at putting green height, severe reductions in creeping bentgrass quality result from ethofumesate treatment. The ethofumesate product label does not allow for applications to putting greens and recommends multiple applications at  $0.84 \text{ kg ha}^{-1}$  every 21 to 28 days for annual bluegrass control in creeping bentgrass as opposed to  $1.13 \text{ kg ha}^{-1}$  every 14 days that was used in this research (Anonymous). Little research is available that examines turfgrass quality in response to amicarbazone; however, there is a body of research that indicates amicarbazone is injurious to creeping bentgrass, which would reduce turfgrass quality. 'L-93' creeping bentgrass was injured 67 and 13% in Indiana and

New Jersey, respectively, following two sequential amicarbazone applications at  $0.2 \text{ kg ha}^{-1}$  applied in the fall 6 to 8 WAIT (McCullough et al. 2010). However, no injury was observed from the same treatments applied in the spring. Single amicarbazone applications at  $0.3$  and  $0.4 \text{ kg ha}^{-1}$  resulted in 16 to 54% creeping bentgrass injury 2 to 6 WAIT (Perry et al. 2009). Since these rates were greater or totaled to a greater amount than in the current research, it is not surprising that turfgrass quality reductions in response to amicarbazone treatment were not observed. There is also little research that examines turfgrass quality in response to methiozolin treatment; however, methiozolin at high rates can be injurious and thus result in turfgrass quality reductions as previously discussed.

Previous research also reported that plant growth regulators had little influence on turfgrass quality when tank-mixed with iron sulfate or ammonium sulfate. Han (2012) examined the interaction of trinexapac-ethyl and flurimidol with iron sulfate and ammonium sulfate for creeping bentgrass/annual bluegrass turfgrass quality. Results indicate that iron sulfate at  $49 \text{ kg Fe ha}^{-1}$  or ammonium sulfate at  $24$  or  $147 \text{ kg N ha}^{-1} \text{ year}^{-1}$  tank-mixed with trinexapac-ethyl or flurprimidol had similar or improved quality relative to the nontreated, however plant growth regulator by fertilizer interactions were rarely significant. In some cases iron sulfate at  $24 \text{ kg N ha}^{-1} \text{ year}^{-1}$  resulted in decreased quality when tank-mixed with fluprimidol; therefore, this research is consistent with previous reports.

*NDVI.* Turfgrass NDVI response to treatment (Table 17) was generally reflective of turfgrass quality (Table 16). Methiozolin alone resulted in similar NDVI response to the nontreated except 6 WAIT in 2014, when response was reduced. Methiozolin combined with iron sulfate resulted in reduced NDVI response relative to the nontreated 4 and 6 WAIT, but a similar response was observed 10 WAIT in 2011-12 and 2014. Methiozolin applied in combination with ammonium

sulfate resulted in similar or improved NDVI response compared to methiozolin alone. This resulted corroborates previous findings, where the addition of ammonium sulfate was found to reduce creeping bentgrass injury (Figure 6). Amicarbazone alone or in combination with ammonium or iron sulfate resulted in similar NDVI response to the nontreated with one exception, when combined with iron sulfate 6 WAIT in 2014 when response was reduced. Ethofumesate containing treatments had reductions in NDVI relative to the nontreated, particularly 4 and 6 WAIT. Reductions in NDVI from ethofumesate containing treatments remained through 10 WAIT in 2014 but recovered in 2011-12 by 10 WAIT. Ammonium sulfate and iron sulfate alone resulted in similar or improved NDVI response to the nontreated at all rating dates and experimental runs.

NDVI response to methiozolin with ammonium sulfate was similar or improved relative to methiozolin alone whereas response with iron sulfate was similar or reduced (Table 17). Amicarbazone applied in combination with ammonium sulfate resulted in similar or improved NDVI response compared to amicarbazone alone, but amicarbazone in combination with iron sulfate resulted in similar, but never improved, NDVI response. Ethofumesate tank-mixed with ammonium sulfate resulted in variable NDVI response relative to ethofumesate alone. Ethofumesate tank-mixed with iron sulfate resulted in similar or reduced NDVI response relative to ethofumesate alone.

Creeping bentgrass NDVI reductions have been previously reported 8 WAIT following four sequential applications of amicarbazone at  $0.049 \text{ g ai ha}^{-1}$  and three sequential applications at  $0.092 \text{ kg ha}^{-1}$  (Jefferies et al. 2013); however, three sequential applications at  $0.065 \text{ kg ha}^{-1}$  did not significantly change NDVI response relative to the nontreated. Han (2012) examined the interaction of plant growth regulators (trinexapac-ethyl and flurimidol) with iron sulfate and

ammonium sulfate for creeping bentgrass/annual bluegrass NDVI response. Results indicate that iron sulfate 12 or 49 kg Fe ha<sup>-1</sup> or ammonium sulfate at 147 kg N ha<sup>-1</sup> year<sup>-1</sup> tank-mixed with trinexapac-ethyl or flurprimidol had similar or improved quality; however, plant growth regulator by ammonium sulfate interactions were rarely significant and plant growth regulator by iron sulfate interactions were never significant.

Visual assessment of turfgrass quality and NDVI were found to have a strong, positive correlation, with a couple exceptions (Table 18). These exceptions occurred when one parameter did not result in a significant model, while the other parameter did. In 2011-12, NDVI analysis did not result in a significant model, while turfgrass quality detected many treatment differences 10 WAIT (Tables 16 and 17). In 2014, turfgrass quality did not result in a significant model 4 WAIT while NDVI analysis detected significant treatment differences. With these exceptions, turfgrass quality and NDVI were positively correlated  $\geq 0.65$  ( $P < 0.001$ ). Previous research indicates that NDVI is highly correlated with visual turfgrass quality assessment (Jefferies et al. 2013; Bremer et al. 2011; Keskin et al. 2008).

Table 13. Annual bluegrass control rated at various weeks after initial treatment (WAIT) from herbicide and iron sulfate tank-mixes from two field experiments conducted in Auburn, AL, in 2010-11 and 2014.

Treatment <sup>a</sup>			2010-11		2014	
Herbicide	Rate	Iron sulfate	12 WAIT <sup>b</sup>	16 WAIT	12 WAIT	16 WAIT
	kg ai ha <sup>-1</sup>	kg Fe ha <sup>-1</sup>	-----%-----			
methiozolin	0.28	---	43 C <sup>c</sup>	43 B	47 C	35 CD
methiozolin	0.56	---	53 B	53 B	65 B	53 BC
methiozolin	1.12	---	100 A	100 A	83 A	83 A
---		0.96	0 E	0 D	0 D	0 E
methiozolin	0.28	0.96	37 D	20 C	33 C	33 CD
methiozolin	0.56	0.96	100 A	100 A	67 B	67 AB
paclobutrazol	0.28	---	100 A	93 A	63 B	23 D
paclobutrazol	0.28	0.96	100 A	100 A	67 B	43 CD
LSD			6.1	13.7	16.2	20.8
Contrast			-----P-value-----			
methiozolin at 0.28 kg ai ha <sup>-1</sup> with versus without iron sulfate			0.035	0.002	0.100	0.867
methiozolin at 0.56 kg ai ha <sup>-1</sup> with versus without iron sulfate			<0.001	<0.001	0.830	0.192
paclobutrazol with versus without iron sulfate			1.000	0.317	0.668	0.058

<sup>a</sup> Treatments were applied twice, sequentially, 24 days apart. Initial treatments were 8 November 2010 and 14 January 2014.

<sup>b</sup> Abbreviations: WAIT, weeks after initial treatment.

<sup>c</sup> Means within a column followed by the same letter do not differ according of Fisher's protected LSD<sub>(0.05)</sub>.

Table 14. Creeping bentgrass injury rated at various weeks after initial treatment (WAIT) from herbicide and iron sulfate tank-mixes from two field experiments conducted in Auburn, AL in 2010-11 and 2014.

Treatment <sup>a</sup>			4 WAIT <sup>b</sup>	8 WAIT	12 WAIT	16 WAIT
Herbicide	Rate	Iron sulfate	-----%-----			
	kg ai ha <sup>-1</sup>	kg Fe ha <sup>-1</sup>				
methiozolin	0.28	---	0 B <sup>c</sup>	0 B	0 B	0 B
methiozolin	0.56	---	0 B	0 B	0 B	0 B
methiozolin	1.12	---	3 AB	7 AB	14 A	9.2 A
---		0.96	0 B	0 B	0 B	0 B
methiozolin	0.28	0.96	0 B	0 B	0 B	0 B
methiozolin	0.56	0.96	0 B	0 B	0 B	0 B
paclobutrazol	0.28	---	5 A	9.2 A	0 B	0 B
paclobutrazol	0.28	0.96	5 A	12 A	0 B	0 B
LSD			4.1	7.2	6.4	5.0

Contrast	-----P-value-----			
methiozolin at 0.28 kg ai ha <sup>-1</sup> with versus without iron sulfate	1.000	1.000	1.000	1.000
methiozolin at 0.56 kg ai ha <sup>-1</sup> with versus without iron sulfate	1.000	1.000	1.000	1.000
paclobutrazol with versus without iron sulfate	1.000	0.489	1.000	1.000

<sup>a</sup> Treatments were applied twice, sequentially, 24 days apart. Initial treatments were 8 November 2010 and 14 January 2014.

<sup>b</sup> Abbreviations: WAIT, weeks after initial treatment.

<sup>c</sup> Means within a column followed by the same letter do not differ according of Fisher's protected LSD<sub>(0.05)</sub>.

Table 15. Annual bluegrass control rated at various weeks after treatment from herbicide and fertilizer tank-mixes from two field experiments conducted in Auburn, AL, in 2011-12 and 2014.

Herbicide <sup>a</sup>	Fertilizer <sup>b</sup>	2011-12					2014				
		4 WAIT <sup>c</sup>	6 WAIT	8 WAIT	10 WAIT	14 WAIT	4 WAIT	6 WAIT	8 WAIT	10 WAIT	14 WAIT
-----%-----											
methiozolin	---	8 CD	7.5 BCD	24 EFG	73 B	98 A	29 A	65 A	78 BC	90 A	95 A
methiozolin	iron sulfate	0 D	0 D	28 CDE	89 A	99 A	7.5 CD	46 B	78 BC	88 A	92 A
methiozolin	ammonium sulfate	5 CD	8.8 BC	43 BC	73 B	100 A	7.5 CD	24 C	64 C	55 B	60 B
amicarbazone	---	9 BC	8.8 BC	25 C-F	0 C	70 BC	0 D	18 CD	44 D	30 C	18 C
amicarbazone	iron sulfate	3 CD	2.5 CD	13 EFG	5 C	79 BC	5 CD	5 DE	5 E	13 D	15 C
amicarbazone	ammonium sulfate	0 D	0 D	23 DEF	0 C	60 C	0 D	5 DE	10 E	0 D	0 C
ethofumesate	---	15 B	15 B	31 CD	88 A	99 A	26 AB	66 A	83 AB	91 A	85 A
ethofumesate	iron sulfate	8 C	7.5 BCD	58 AB	93 A	98 A	13 CD	53 AB	95 A	99 A	88 A
ethofumesate	ammonium sulfate	36 A	43 A	63 A	90 A	84 AB	15 BC	45 B	89 AB	99 A	90 A
---	iron sulfate	0 D	0 D	7.5 GF	0 C	0 D	2.5 CD	0 E	0 E	0 D	0 C
---	ammonium sulfate	0 D	0 D	0 G	0 C	0 D	2.5 CD	0 E	0 E	0 D	0 C
LSD		6.9	7.7	18.6	7.2	16.7	13.5	15.8	15.1	12.7	18.0

<sup>a</sup> Herbicides were applied at 0.84, 0.07, and 1.13 kg ai ha<sup>-1</sup> for methiozolin, amicarbazone, and ethofumesate, respectively. All treatments were applied sequentially every 2 weeks for a total of four applications. Initial application dates were 1 November 2011 and 15 January 2014.

<sup>b</sup> Fertilizers were tank-mixed with herbicides where appropriate and applied at 0.96 kg Fe ha<sup>-1</sup> and 12.2 kg N ha<sup>-1</sup> for iron sulfate and ammonium sulfate, respectively.

<sup>c</sup> Abbreviations: WAIT, weeks after initial treatment.

<sup>d</sup> Means within a column followed by the same letter do not differ according of Fisher's protected LSD<sub>(0.05)</sub>.

Table 16. Turfgrass quality rated at various weeks after treatment from herbicide and fertilizer tank-mixes from two field experiments conducted in Auburn, AL, in 2011-12 and 2014.

Herbicide <sup>a</sup>	Fertilizer <sup>b</sup>	2011-12					2014				
		4 WAIT <sup>c</sup>	6 WAIT	8 WAIT	10 WAIT	14 WAIT	4 WAIT	6 WAIT	8 WAIT	10 WAIT	14 WAIT
---	---	6.8 ABC <sup>d</sup>	6.8 A-D	6.5 AB	5.8 CD	5.0 BC	5.0 A	5.8 CDE	6.3 ABC	6.3 B-E	6.3 AB
methiozolin	---	6.0 CD	6.3 CD	4.8 CD	4.8 DE	5.5 ABC	4.5 AB	5.3 EF	5.8 C	5.8 DE	6.3 AB
methiozolin	iron sulfate	6.3 BCD	5.8 DE	4.0 D	4.3 EF	5.0 BC	4.8 AB	6.0 CDE	6.0 BC	5.5 EF	5.8 AB
methiozolin	ammonium sulfate	7.3 AB	6.8 A-D	5.8 BC	6.0 BC	6.8 A	4.8 AB	6.3 CD	6.5 ABC	6.5 BCD	6.8 A
amicarbazone	---	6.8 ABC	6.5 BCD	5.8 BC	6.5 ABC	6.3 AB	5.3 A	5.5 DE	6.0 BC	6.0 CDE	7.0 A
amicarbazone	iron sulfate	7.0 ABC	7.3 ABC	6.5 AB	7.0 AB	6.5 A	5.0 A	6.5 BC	6.8 AB	6.8 BC	6.0 AB
amicarbazone	ammonium sulfate	7.5 A	7.8 A	7.3 A	7.5 A	6.3 AB	4.5 AB	7.3 AB	7.0 A	7.0 AB	6.8 A
ethofumesate	---	5.5 D	5.0 E	4.0 D	3.3 FG	3.3 E	4.0 B	3.8 G	2.5 DE	1.3 F	3.8 C
ethofumesate	iron sulfate	4.0 E	3.8 F	2.5 E	2.3 GH	3.5 DE	4.5 AB	4.5 FG	2.0 E	1.8 F	3.8 C
ethofumesate	ammonium sulfate	4.0 E	3.0 F	1.3 E	1.3 H	2.3 E	4.5 AB	4.5 FG	3.0 D	2.0 F	5.0 BC
---	iron sulfate	6.8 ABC	7.3 ABC	6.5 AB	6.0 BC	5.0 BC	5.0 A	6.3 CD	6.8 AB	6.5 BCD	6.3 AB
---	ammonium sulfate	7.5 A	7.5 AB	7.0 AB	6.3 BC	4.8 CD	4.8 AB	7.5 A	7.0 A	7.8 A	6.8 A
LSD		1.09	1.22	1.39	1.15	1.30	0.86; NS	0.88	0.79	0.74	1.33

<sup>a</sup> Herbicides were applied at 0.84, 0.07, and 1.13 kg ai ha<sup>-1</sup> for methiozolin, amicarbazone, and ethofumesate, respectively. All treatments were applied sequentially every 2 weeks for a total of four applications. Initial application dates were 1 November 2011 and 15 January 2014.

<sup>b</sup> Fertilizers were tank-mixed with herbicides where appropriate and applied at 0.96 kg Fe ha<sup>-1</sup> and 12.2 kg N ha<sup>-1</sup> for iron sulfate and ammonium sulfate, respectively.

<sup>c</sup> Abbreviations: WAIT, weeks after initial treatment; NS, not significant.

<sup>d</sup> Means within a column followed by the same letter do not differ according of Fisher's protected LSD<sub>(0.05)</sub>.



Table 17. Turfgrass normalized difference vegetative index (NDVI) rated at various weeks after treatment from herbicide and fertilizer tank-mixes from two field experiments conducted in Auburn, AL, in 2011-12 and 2014.

Herbicide <sup>a</sup>	Fertilizer <sup>b</sup>	2011-12			2014		
		4 WAIT <sup>c</sup>	6 WAIT	10 WAIT	4 WAIT	6 WAIT	10 WAIT
-----%-----							
---	---	0.807 BCD <sup>d</sup>	0.787 ABC	0.689 B	0.694 AB	0.755 AB	0.710 AB
methiozolin	---	0.805 B-E	0.777 BCD	0.785 AB	0.667 BCD	0.691 E	0.655 B
methiozolin	iron sulfate	0.787 EF	0.742 EF	0.745 AB	0.656 CD	0.691 E	0.665 AB
methiozolin	ammonium sulfate	0.818 AB	0.783 BCD	0.732 AB	0.687 ABC	0.746 ABC	0.704 AB
amicarbazone	---	0.808 BC	0.777 BCD	0.777 AB	0.690 ABC	0.731 BC	0.687 AB
amicarbazone	iron sulfate	0.797 CDE	0.768 CD	0.726 AB	0.696 AB	0.723 CD	0.687 AB
amicarbazone	ammonium sulfate	0.818 AB	0.787 ABC	0.698 B	0.709 A	0.770 A	0.728 A
ethofumesate	---	0.789 DEF	0.761 DE	0.759 AB	0.639 D	0.667 EF	0.459 D
ethofumesate	iron sulfate	0.773 F	0.722 F	0.797 AB	0.638 D	0.652 F	0.462 D
ethofumesate	ammonium sulfate	0.739 G	0.650 G	0.813 A	0.682 ABC	0.696 DE	0.554 C
---	iron sulfate	0.810 ABC	0.794 AB	0.758 AB	0.700 AB	0.730 BC	0.701 AB
---	ammonium sulfate	0.827 A	0.807 A	0.722 AB	0.691 ABC	0.770 A	0.727 A
LSD		0.0189	0.0227	0.1093; NS	0.0371	0.0299	0.0656

<sup>a</sup> Herbicides were applied at 0.84, 0.07, and 1.13 kg ai ha<sup>-1</sup> for methiozolin, amicarbazone, and ethofumesate, respectively. All treatments were applied sequentially every 2 weeks for a total of four applications. Initial application dates were 1 November 2011 and 15 January 2014.

<sup>b</sup> Fertilizers were tank-mixed with herbicides where appropriate and applied at 0.96 kg Fe ha<sup>-1</sup> and 12.2 kg N ha<sup>-1</sup> for iron sulfate and ammonium sulfate, respectively.

<sup>c</sup> Abbreviations: WAIT, weeks after initial treatment; NS, not significant.

<sup>d</sup> Means within a column followed by the same letter do not differ according of Fisher's protected LSD<sub>(0.05)</sub>.

Table 18. Pearson's correlation coefficients between turfgrass quality and normalized difference vegetative index (NDVI) from two field experiments conducted in Auburn, AL in 2011-12 and 2014.

Year	WAIT <sup>a</sup>	coefficient	P-value
2011-12	4	0.77	<0.001
	6	0.79	<0.001
	10	-0.45	1.000
2014	4	0.30	0.035
	6	0.65	<0.001
	10	0.88	<0.001

<sup>a</sup> Abbreviation: WAIT, weeks after initial treatment.

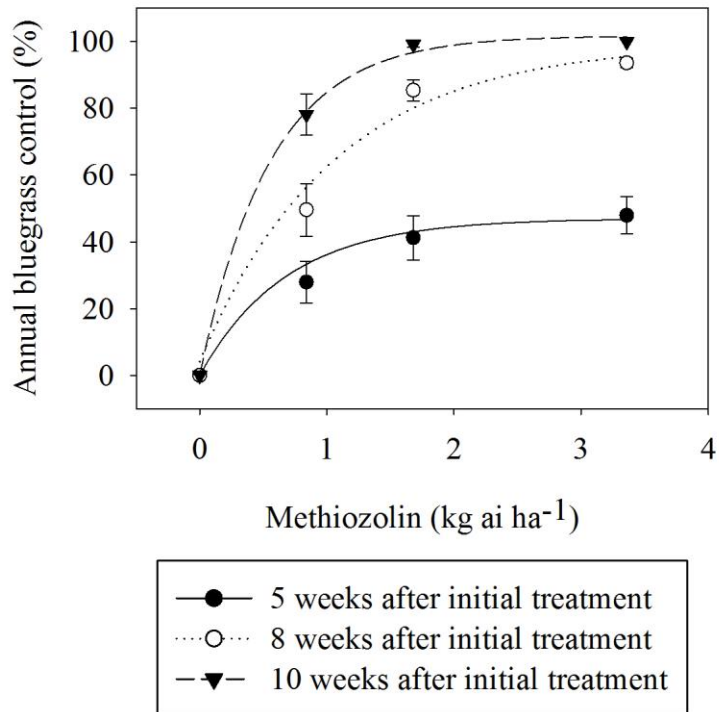


Figure 5. Annual bluegrass control from methiozolin applied three times sequentially at 0.84, 1.68, and 3.36 kg ai ha<sup>-1</sup> from two combined field experiments in Auburn, AL in 2012 and 2014. Each rate was applied with and without ammonium sulfate at 12.2 kg N ha<sup>-1</sup>, but data were pooled as this effect was not significant. Points are means surrounded by their standard error. Lines represent nonlinear regression with the model  $\text{control} = Y_0 + a(1 - e^{-b \cdot \text{rate}})$ .  $Y_0 = -0.09, 4.11, \text{ and } 0.00$ ,  $a = 49.98, 95.29, \text{ and } 101.8$ , and  $b = 1.00, 0.95, \text{ and } 1.79$  for 5, 8 and 10 weeks after initial treatment, respectively.

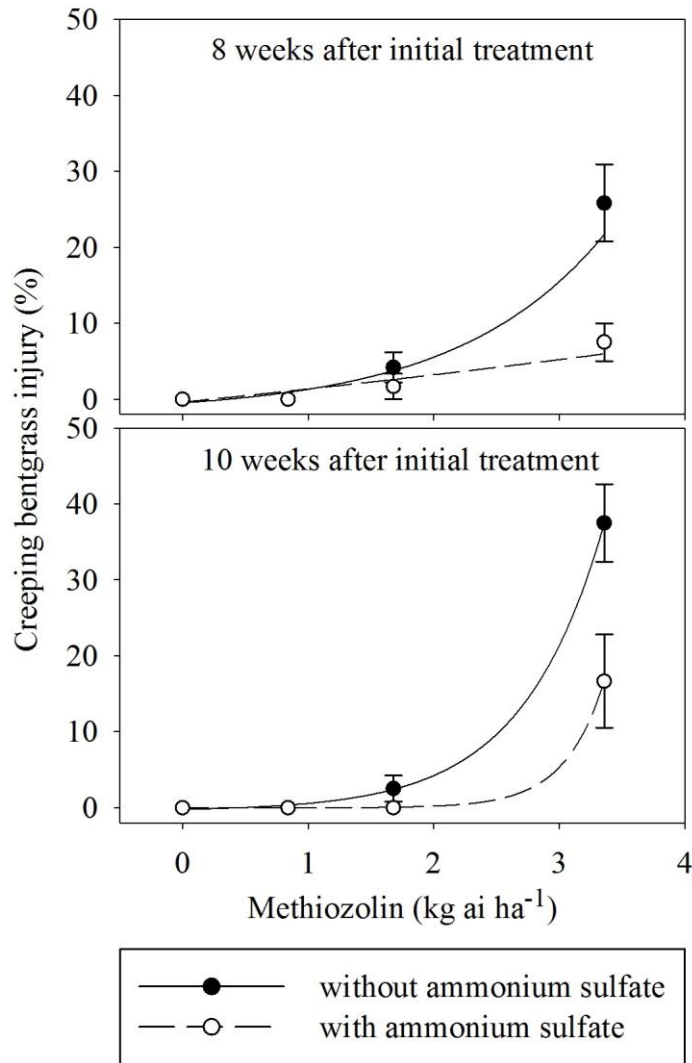


Figure 6. Creeping bentgrass injury from methiozolin applied three times sequentially at 0.84, 1.68, and 3.36 kg ai ha<sup>-1</sup> with and without ammonium sulfate at 12.2 kg N ha<sup>-1</sup> from two combined field experiments in Auburn, AL in 2012 and 2014. Points are means surrounded by their standard error. Lines represent nonlinear regression with the model injury =  $Y_0 + a * e^{b * \text{rate}}$ . Nonlinear regression models are injury =  $-1.82 + 1.32 * e^{0.86 * \text{rate}}$  for 8 WAIT without ammonium sulfate, injury =  $-23.98 + 23.57 * e^{0.07 * \text{rate}}$  for 8 WAIT with ammonium sulfate, and injury =  $-0.45 + 0.21 * e^{1.54 * \text{rate}}$  for 10 WAIT without ammonium sulfate and injury =  $-0.03 + 0.0004 * e^{0.18 * \text{rate}}$  for 10 WAIT with ammonium sulfate (note that this model did not satisfy convergence criteria).

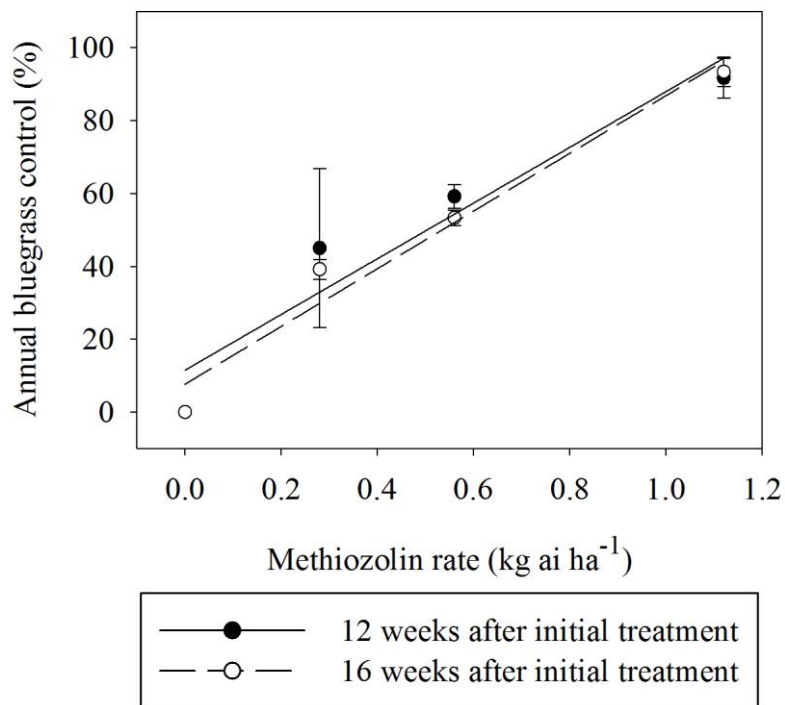


Figure 7. Annual bluegrass control from methiozolin applied three times sequentially at 0.84, 1.68, and 3.36 kg ai ha<sup>-1</sup> from two combined field experiments in Auburn, AL in 2010-11 and 2014. Points are means surrounded by their standard error. Lines represent simple linear regression with the models control = 11.50 + 76.45 (rate) for 12 weeks after initial treatment (WAIT) and control = 7.67 + 79.17(rate) for 16 WAIT.

## **Broad Implications for Methiozolin Field Application**

**Areas of Use.** Methiozolin is a herbicide that is being evaluated for registration in the United States to control annual bluegrass (*Poa annua*) in golf course putting greens, including both creeping bentgrass (*Agrostis stolonifera*) and ultra-dwarf bermudagrass (*Cynodon dactylon* × *C. transvalensis*). It is currently registered for use on golf courses in South Korea and Japan. United States Golf Association (USGA) specifications require a sand-based root-zone (USGA Green Section 2004). Sandy soils are characterized as having small amounts of herbicide adsorption and a relatively high propensity for leaching (Harris 1966; Harris and Sheets 1965; Nearpass 1965; Obrigawitch et al. 1981; Stougaard et al. 1990; Talbert and Fletchall 1965; Upchurch and Mason 1962; Weber et al. 1969). Alternatively, golf course facilities with limited budgets will construct so-called “push-up” greens where root-zones are simply the native soil or sand mixed with the native soil (USGA Green Section 2004). The native soil component of these push-up greens will generally have higher clay and organic matter content than the USGA specified sand-based alternative, which results in greater herbicide adsorption and less propensity for leaching among other factors. Therefore, sand-based soil bias of this research is well founded due to economic and agronomic factors.

Research in Chapter 5 indicates that while sand-based root-zones present a “worst-case-scenario” in terms of herbicide movement, movement of methiozolin will likely be a nonissue for turfgrass managers.

**Application Techniques.** While application techniques were not a direct part of this research, this research offers implications for methiozolin field application. Previous research indicates that fall application timing is more efficacious for annual bluegrass control than spring application timing (Brosnan et al. 2013; McCullough and Gómez de Barreda 2012; Trappe et al. 2012). This finding is logical as annual bluegrass germinates in the fall, with a second flush of germination in the spring (McElroy et al. 2004). Fall applications generally target a smaller, less mature plant which results in more efficacious control. Research findings in Chapter 2 indicate that smaller plants were better controlled than larger plants (Tables 2 and 3). Application timing may not be as important for the perennial biotype of annual bluegrass, but research thus far has been limited to the annual biotype of annual bluegrass.

Previous research also indicates that multiple, sequential applications have been found to be more efficacious for annual bluegrass control than a single application. Research indicates that a minimum of three applications totaling 3.36 kg ai ha<sup>-1</sup> are necessary for annual bluegrass control with minimal creeping bentgrass injury (McCullough and Gómez de Barreda 2012; McCullough et al. 2013).

The research in Chapter 2 supports that multiple methiozolin applications are necessary, as a single application did not result in complete annual bluegrass control regardless of growth stage or application placement at the 3.36 kg ai ha<sup>-1</sup> rate (Table 3); however, smaller plants were better controlled than larger plants (Table 2).

Multiple methiozolin applications lead to a longer residual in the soil compared to a single dose at comparable rates, resulting in long PRE annual bluegrass control. Extended PRE control is important because the research indicates that PRE control from methiozolin is much shorter than the typical annual bluegrass germination period. Experiments indicate that

methiozolin had PRE activity until 25 DAT (Chapter 2). Previous research indicates barnyardgrass was controlled until 6 weeks after application (Norsworthy et al. 2011). Other researchers also reported PRE annual bluegrass control but did not disclose the duration of control (Hwang and Koo 2009; Nam et al. 2012).

The mode-of-action of methiozolin is under debate (Grossmann et al. 2012; Lee et al. 2007); therefore the site-of-action is also largely unknown. Grossmann et al. (2012) propose that the mode-of-action is tyrosine aminotransferase (TAT) inhibition. TAT is located in the chloroplast, which makes the leaves the desired location for methiozolin exposure (Soll et al. 1985). Lee et al. (2007) propose that methiozolin inhibits cell wall biosynthesis via inhibiting glucose incorporation into hemicellulose and cellulose, which makes the meristems (crown and root tips) the desired location for methiozolin exposure.

Some researchers have reported that applying a small amount of irrigation following methiozolin application (so-called “watering in”) results in more efficacious annual bluegrass control (R.H. Walker, personal communication). Research findings in Chapter 2 would suggest that watering in could be helpful if delayed until 24 hours after treatment. Delaying allows for maximum foliar absorption, which was found to be complete 24 hours after treatment (Table 5). After 24 hours, washing the remaining methiozolin into the root-zone may encourage further absorption via the roots and thus enhance overall control. This premise relies on the assumption that more methiozolin into the plant will result in more control, regardless of point-of-entry into the plant. Translocation is limited within annual bluegrass (Tables 5 and 6; Figure 2); therefore, if the site-of-action cannot be reached from a particular point-of-entry than there is no need to target that point-of-entry. Translocation only occurs with the evapotranspiration stream in the plant; basipetal translocation was found to be negligible. If the site-of-action is the crown, then



foliar absorption will not result in methiozolin reaching the site-of-action and only root absorption should be targeted during application.

**Cultural Practices to Enhance Annual Bluegrass Control with Methiozolin.** Since methiozolin does not readily desorb from soil once adsorbed (Table 11), turfgrass managers are likely free to irrigate as they so choose. As previously discussed, one exception would be the 24 hour period directly after application, which accounts for maximum foliar absorption of methiozolin (Table 5). Some researchers have reported that methiozolin phytotoxicity increases in wet or flooded areas (J. Baird, personal communication). Therefore, future research is needed in this area.

Mowing with clippings collected may effectively remove methiozolin from the system, as translocation is toward the leaf tip (Tables 5 and 6; Figure 2). The hydrophobic octanol-water partitioning coefficient ( $\log K_{ow} = 3.9$ ) strongly suggests that once absorbed, methiozolin is unlikely to desorb from plant material similar to soil sorption and desorption findings (Chapter 3) (Anonymous 2012b). Therefore, returning the clippings to the system will not likely result in any appreciable methiozolin returns to the system in a plant-available form. Future research is necessary to determine the effects of clipping return or removal.

Proper management of a sand-based root-zone requires frequent sand top-dressings to dilute organic matter accumulation from dead and decaying rhizomes and other plant parts. Findings in this research highlight the importance of this practice as a thick organic matter layer may prevent roots from being exposed to methiozolin due to the strong adsorptive nature of organic matter for methiozolin (Figure 8). Other practices that diminish organic matter accumulation such as vertical mowing and aerification are also important. Organic matter

accumulation can be significant and will influence the amount of methiozolin available for root uptake, as observed in Chapter 3 (Table 8).



Figure 8. Profiles of United States Golf Association (USGA) specified root-zones for putting greens. The profile on the left exhibits organic matter accumulation in the upper zone, characterized by the darker brown color. The profile on the right does not show signs of organic matter accumulation.

Practices that inhibit annual bluegrass growth and germination should be combined with methiozolin application for a more integrated approach to weed control. These approaches are outlined in Chapter 1, but one area deserves additional consideration. Previous research reports that annual bluegrass populations are best managed (reduced) with limited P applications and soil pH approximately 5.0, provided the desirable turfgrass is tolerant to these practices (Juska and Hanson 1969; Kuo 1993; Varco and Sartain 1966). Research in Chapter 4 indicates that soil

pH had no appreciable influence on methiozolin soil sorption. Therefore, turfgrass managers can manage soil pH freely to decrease annual bluegrass populations in conjunction with methiozolin application.

**Herbicide Resistance Mitigation.** As documented in Chapter 1, annual bluegrass is resistant to many herbicide modes-of-action. Typically, herbicide resistance develops when a herbicide is exclusively and repeatedly used (Gressel et al. 1996; Norsworthy et al. 2012; Owen 2008). The extremely limited options for annual bluegrass control in creeping bentgrass and ultra-dwarf bermudagrass putting greens (documented in Chapter 1) suggest that turfgrass managers will likely use this herbicide exclusively. If methiozolin is exclusively and repeatedly used, annual bluegrass will develop resistance to methiozolin.

To mitigate annual bluegrass resistance to methiozolin, rotation on herbicide modes-of-action can effectively vary the selection pressure and reduce the chances of resistance development to null (Gressel and Segel 1990; Gressel et al. 1996; Norsworthy et al. 2012). However, with very limited herbicide options available to turfgrass managers (documented in Chapter 1), rotating modes-of-action is functionally impossible.

## Literature Cited

- Adams, F., C. Burmester, N. V. Hue, and L. F. Long. 1982. A comparison of column displacement and centrifuge methods of obtaining soil solution. *Soil Sci. Soc. Am. J.* 44:733–735.
- Aldahir, P. C. F., J. S. McElroy, and M. L. Flessner. 2011. Annual bluegrass control in bermudagrass overseeded with glyphosate tolerant perennial ryegrass. *Proc., Crop Sci. Soc. of Amer.* No. 330–2.
- Aldahir, P. C. F., J. S. McElroy, M. L. Flessner, J. D. McCurdy. 2012. Glyphosate rates and timing for annual bluegrass control in bermudagrass overseeded with glyphosate tolerant perennial ryegrass. *Proc. SIGRA Brazilian Turfgrass Sym. (Botucatu, Brazil)* 6:CD-ROM. ISSN 21776691.
- Al-Rajab, A. J., S. Amellal, and M. Schiavon. 2008. Sorption and leaching of  $^{14}\text{C}$ -glyphosate in agricultural soils. *Agron. Sustain. Dev.* 28:419–428.
- Anonymous. Prograss® herbicide product label. Bayer Publication No. 4193821. Research Triangle Park, NC: Bayer Environmental Science. 6 p.
- Anonymous. 2009a. Bensumec™ 4 LF herbicide product label. PBI/Gordon Publication No. 845/8-2009. Kansas City, MO:PBI/Gordon Corporation. 3 p.
- Anonymous. 2009b. Quali-Pro Rimsulfuron 25 DF herbicide product label. Makhteshim Agan of North America, Inc. Publication No. 12914. 27 p.
- Anonymous. 2010. Rubigan® A. S. T&O supplemental product label. Gowan Publication No. 01-R1110. Yuma, AZ: Gowan Company, L.L.C. 2 p.
- Anonymous. 2012a. Herbicide resistant annual bluegrass globally. Available at <http://www.weedscience.org/In.asp>. Accessed June 5, 2012.

- Anonymous. 2012b. Methiozolin 25%EC material safety data sheet. Moghu Research Center Ltd. Publication No. MTZ\_v08. Daejeon, Korea. 4 p.
- Baldock, J. A. and P.N. Nelson. 2000. Soil Organic Matter. Section B Pages 25–84 in M. E. Sumner, eds. Handbook of Soil Science. Boca Raton, FL: CRC Press LLC.
- Baldwin, N. A. 1993. Chemical control of *Poa annua*: a review. J. Sports Turf Res. Inst. 69:7–19.
- Beam, J. B. and S. D. Askew. 2007. Fate of prohexadione calcium in annual bluegrass (*Poa annua*) and three turfgrasses. Weed Sci. 55:541–545.
- Beard, J. B. 1970. An ecological study of annual bluegrass. USGA Green Sect. Rec. 8:13–18.
- Beard, J. B. 1973. Turfgrass: Science and Culture. Englewood Cliffs, NJ: Prentice-Hall. 658 p.
- Beard, J. B., P. E. Rieke, A. J. Turgeon, and J. M. Vargas. 1978. Annual bluegrass (*Poa annua* L.) description, adaptation, culture and control. Res. Rep. 352. Michigan State Univ. Agric. Exp. Stn., East Lansing, MI.
- Best, J. A. and J. B. Weber. 1974. Disappearance of *s*-triazines as affected by soil pH using a balance-sheet approach. Weed Sci. 22:364–373.
- Best, J. A. and J. B. Weber. 1975. Influence of soil pH on *s*-triazine availability to plants. Weed Sci. 23:378–382.
- Bingham, S. W., R. E. Schmidt, and C. K. Curry. 1969. Annual bluegrass control in overseeded bermudagrass putting green turf. Agron. J. 61:908–911.
- Bingham, S. W. and R. L. Shaver. 1979. Effectiveness of herbicide programs for annual bluegrass (*Poa annua*) control in bermudagrass (*Cynodon dactylon*). Weed Sci. 27:367–370.

- Binkholder, K. M., B. S. Fresenburg, T. C. Tueton, X. Xiong, and R. J. Smeda. 2011. Selection of glyphosate-resistant annual bluegrass (*Poa annua*) on a golf course. *Weed Sci.* 59:286–289.
- Böger, P, B. Matthes, and J. Schmalfuss. 2000. Towards the primary target of chloroacetamids- new findings pave the way. *Pest. Manag. Sci.* 56:497–508.
- Bremer, D. J., H. Lee, K. Su, and S. J. Keeley. 2011b. Relationships between normalized difference vegetation index and visual quality in cool-season turfgrass, II: factors affecting NDVI and its component reflectances. *Crop Sci.* 51:2219–2227.
- Briggs, G. G., R. H. Bromilow, and A. A. Evans. 1982. Relationships between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. *Pestic. Sci.* 13:495–504.
- Bristow, C., J. S. McElroy, and E. A. Guertal. 2011. Utilization of zinc sulfate for *Poa annua* control. *Proc., Crop Sci. Soc. of Amer.* No. 229–4.
- Brosnan, J. T., A. W. Thoms, P. E. McCullough, G. R. Armel, G. K. Breeden, J. C. Sorochan, and T. C. Mueller. 2010. Efficacy of flazasulfuron for control of annual bluegrass (*Poa annua*) and perennial ryegrass (*Lolium perenne*) as influenced by nitrogen. *Weed Sci.* 58:449–456.
- Brosnan, J. T., G. K. Breeden, P. E. McCullough, and G. M. Henry. 2012a. PRE and POST control of annual bluegrass (*Poa annua*) with indaziflam. *Weed Technol.* 26:48–53.
- Brosnan, J.T., G.K. Breeden, and T.C. Mueller. 2012b. A glyphosate-resistant biotype of annual bluegrass in Tennessee. *Weed Sci.* 60:97–100.

- Brosnan, J. T., G. M. Henry, G. K. Breeden, T. Cooper, and T. J. Serensits. 2013a. Methiozolin efficacy for annual bluegrass control on sand- and soil-based creeping bentgrass putting greens. *Weed Technol.* 27:310–316.
- Brosnan, J. T., S. Calvache, G. K. Breeden, and J. C. Sorochan. 2013b. Rooting depth, soil type, and application rate effects on creeping bentgrass injury with amicarbazone and methiozolin. *Crop Sci.* 53:655–659.
- Brusseau, M. L., R. E. Jessup, and P. S. C. Rao. 1991. Nonequilibrium sorption of organic chemicals: elucidation of rate-limiting processes. *Environ. Sci. Technol.* 25:134–142.
- Callahan, L. M. and E. R. McDonald. 1992. Effectiveness of bensulide in controlling two annual bluegrass (*Poa annua*) subspecies. *Weed Technol.* 6:97–103.
- Coats, G. E. and J. V. Krans. 1986. Evaluation of ethofumesate for annual bluegrass (*Poa annua*) and turfgrass tolerance. *Weed Sci.* 34:930–935.
- Cutulle, M. A., J. S. McElroy, R. W. Millwood, J. C. Sorochan, and C. N. Stewart. 2009. Selection of bioassay method influences detection of annual bluegrass resistance to mitotic-inhibiting herbicides. *Crop Sci.* 49:1088–1095.
- Dest, W. M. and D. W. Allinson. 1981. Influence of nitrogen and phosphorus fertilization on the growth and development of *Poa annua* L. (annual bluegrass). *Proc. Second Intl. Turfgrass Res. Conf.* Pp. 325–332.
- Dickens, R. 1979. Control of annual bluegrass (*Poa annua*) in overseeded bermudagrass (*Cynodon* spp.) golf greens. *Weed Sci.* 27:642–644.
- Elford, E. M. A., F. J. Tardif, D. E. Robinson, and E. M. Lyons. 2008. Effect of perennial ryegrass overseeding on weed suppression and sward competition. *Weed Technol.* 22:231–239.

- Farmer, W. J. and Y. Aochi. 1974. Picloram sorption by soils. *Soil Sci. Soc. Amer. Proc.* 38:418–423.
- Fearis, D. W. 2002. The dos and don'ts of tank-mixing herbicides and fertilizers. *Grounds Maintenance*. April 2002.
- Flessner, M. L. and J. S. McElroy. 2011. Timing of flumioxazin application for annual bluegrass control in dormant bermudagrass. *Proc., Crop Sci. Soc. of Amer.* No. 330–3.
- Flessner, M.L., J.S. McElroy, and G.R. Wehtje. 2014. Glyphosate for annual bluegrass (*Poa annua*) control in glyphosate-tolerant perennial ryegrass overseeding. *Weed Technol.* 28:213–224.
- Gardner, D. S., T. K. Danneberger, and E. K. Nelson. 2004. Lateral spread of glyphosate-resistant transgenic creeping bentgrass (*Agrostis stolonifera*) lines in established turfgrass swards. *Weed Technol.* 18:773–778.
- Gaussoin, R. E and B. E. Branham. 1989. Influence of cultural factors on species dominance in a mixed stand of annual bluegrass/creeping bentgrass. *Crop Sci.* 29:480–484.
- Gevao, B. K. T. Semple, and K. C. Jones. 2000. Bound pesticide residues in soils: a review. *Environmental Pollution* 108:3–14.
- Gibeault, V. A. 1966. Annual meadow-grass—a major weed of fine turf. *J. Sports Turf Res. Inst.* 41:48–52.
- Gibeault, V. A. and N. R. Goetze. 1972. Annual meadow-grass. *J. Sports Turf Res. Inst.* 48:1–11.
- Goddard, M. J. R. 2009. Physiological and environmental basis for turfgrass and weed response to mesotrione formulations. Ph.D dissertation. Blacksburg, VA: Virginia Polytechnic Institute and State University. 127p.



- Goetz, A. J., G. Wehtje, R. H. Walker, and B. Hajek. 1986. Soil solution and mobility characterization of imazaquin. *Weed Sci.* 34:788–793.
- Goetz, A. J., G. Wehtje, R. H. Walker, and B. Hajek. 1989. Sorption and mobility of chlorimuron in Alabama soils. *Weed Sci.* 37:428–433.
- Goss, R. L. 1964. Preemergence control of annual bluegrass (*Poa annua* L.). *Agron. J.* 56:479–481.
- Greene, S. W. and D. W. H. Walton. 1975. An annotated check list of the sub-Antarctic and Antarctic vascular flora. *Polar Rec.* 17: 473–484.
- Gressel, J., L. Segel, and J. K. Ransom. 1996. Managing the delay of evolution of herbicide resistance in parasitic weeds. *Int. J. Pest Manag.* 42:113–129.
- Gressel, J. and L. A. Segel. 1990. Modelling the effectiveness of herbicide rotations and mixtures as strategies to delay or preclude resistance. *Weed Technol.* 4:186–198.
- Grey, T. L., G. R. Wehtje, B. F. Hajek, and R. H. Walker. 1996. Sorption and mobility of bentazon in coastal plain soils. *Weed. Sci.* 44:166–170.
- Grey, T.L., R. H. Walker, G. R. Wehtje, and H. G. Hancock. 1997. Sulfentrazone adsorption and mobility as affected by soil and pH. *Weed Sci.* 45:733–738.
- Grossmann, K., J. Hutzler, S. Tresch, N. Christiansen, R. Looser, and T. Ehrhardt. 2011. On the mode of action of the herbicides cinmethylin and 5-benzyloxymethyl-1,2-isoxazolines: putative inhibitors of plant tyrosine aminotransferase. *Pest. Manag. Sci.* 68:482–492.
- Haguewood, J. B., J. Moss and X. Xiong. 2012. Efficacy and safety of methiozolin for pre-emergence control of annual bluegrass (*Poa annua* L.) on creeping bentgrass (*Agrostis stolonifera* L.) putting greens. *Proc Crop Sci. Soc. of Amer.* 105–27.

- Han, K. M. 2012. Influence of cultural and chemical management strategies on annual bluegrass populations on golf course putting greens. M.S. Thesis. State College, PA: The Pennsylvania State University. 186 p.
- Han, K. M. and J. Kaminski. 2011. Control of perennial annual bluegrass biotypes with postemergence herbicides. Proc., Crop Sci. Soc. of Amer. No. 104–30.
- Han, K. M. and J. Kaminski. 2012. Influence of methiozolin rates and application timings on *Poa annua* populations. Proc Crop Sci. Soc. of Amer. 366–25.
- Hanson, B. D. and C. A. Mallory-Smith. 2000. Diuron-resistant *Poa annua* is resistant to norflurazon. Weed Sci. 48:666–668.
- Harris, C. I. 1966. Adsorption, movement, and phytotoxicity of monuron and s-triazine herbicides in soil. Weeds 14:6–10.
- Harris, C. I. 1967. Movement of herbicides in soil. Weeds 15:214–216.
- Harris, C. I. and T. J. Sheets. 1965. Influence of soil properties on adsorption and phytotoxicity of CIPC, diuron, and simazine. Weeds 13:215–219.
- Harrison, G. W. J. B. Weber, and J. V. Barid. 1976. Herbicide phytotoxicity as affected by selected properties of North Carolina soils. Weed Sci. 24:120–126.
- Heap, I. M. 1997. The occurrence of herbicide-resistant weeds worldwide. Pestic. Sci. 51:235–243.
- Hess, F. D and C. L. Foy. 2000. Interaction of surfactants with plant cuticles. Weed Technol. 14:807–813.
- Heim, D. R., J. R. Skomp, E. E. Tschabold, and I. M. Larrinua. 1990. Isoxaben inhibits the synthesis of acid insoluble cell wall materials in *Arabidopsis thaliana*. Plant Physiol. 93:695–700.

- Helling, C. S and B. C. Turner. 1968. Pesticide mobility: determination by soil thin-layer chromatography. *Science*. 162:562–563.
- Helling, C. S. 1971. Pesticide mobility in soils. II. Applications of soil thin-layer chromatography. *Soil Sci. Soc. Amer. Proc.* 35:737–743.
- Hiltbold, A. E. and G. A. Buchanan. 1977. Influence of soil pH on persistence of atrazine in the field. *Weed. Sci.* 25:515–520.
- Holm, L., J. Doll, E. Holm, J. Panchho, and J. Herberger. 1997. *World weeds, natural histories and distribution*. New York: J. Wiley. 1129 p.
- Hoyle, J. A., C. Straw, G. Henry, T. Cooper, L. Beck, J. Brosnan, and G. Breeden. 2012. Fall applications of methiozolin for the control of annual bluegrass in sand-based and push-up greens. *Proc. Crop. Sci. Soc. of Amer.* 254–9.
- Hsu, F. C., R. L. Marxmiller, and A. Y. S. Yang. 1990. Study of root uptake and xylem translocation of cinmethylin and related compounds in detopped soybean roots using a pressure chamber technique. *Plant Physiol.* 93:1573–1578.
- Hutto, K. C., G. E. Coats, and J. M. Taylor. 2004. Annual bluegrass (*Poa annua*) resistance to simazine in Mississippi. *Weed Technol.* 18:846–849.
- Hwang, I. T., H. R. Kim, D. J. Jeon, K. S. Hong, J. H. Song, and K. Y. Cho. 2005. 5-(2,6-Difluorobenzyl)oxymethyl-5-methyl-3-(3-methylthiophen-2-yl)-1,2-isoxazoline as a useful rice herbicide. *J. Agric. Food Chem.* 53:8639–8643.
- Hwang, K. and S. Koo. 2009. Herbicidal activity of MRC-01 on *Poa annua*. *Proc. South Weed Sci. Soc.* 62:194.

- Hwang, K. H., J. S. Lim, S. H. Kim, H. R. Chang, K. Kim, S. J. Koo, and J. H. Kim. 2013. Soil metabolism of [<sup>14</sup>C]methiozolin under aerobic and anaerobic flooded conditions. *J. Ag. Food Chem.* 61:6799–6805.
- Isgrigg, J., F. H. Yelverton, C. Brownie, and L. S. Warren. 2002. Dinitroaniline resistant annual bluegrass in North Carolina. *Weed Sci.* 50:86–90.
- Itoh, M., H. Kobayashi, and K. Ueki. 1997. Variation in seed germination and dormancy of *Poa annua* L. in golf course. *Grassland Sci. (Japan)*. 42:299–306.
- Jacques, G. L. and R. G. Harvey. 1979. Adsorption and diffusion of dinitroaniline herbicides in soils. *Weed Sci.* 27:450–455.
- Jamet, P. and J. C. Thoisy-Dur. 1988. Pesticide mobility in soils: assessment of the movement of isoxaben by soil thin-layer chromatography. *Bull. Environ. Contam. Toxicol.* 41:135–142.
- Jefferies, M. D., F. H. Yelverton, and T. W. Gannon. 2013. Annual bluegrass (*Poa annua*) control in creeping bentgrass putting greens with amicarbazone and paclobutrazol. *Weed Technol.* 27:520–526.
- Johnson, B. J. 1980. Postemergence winter weed control in bermudagrass (*Cynodon dactylon*) Turf. *Weed Sci.* 28:385–392.
- Johnson, B. J. 1983. Response to ethofumesate of annual bluegrass (*Poa annua*) and overseeded bermudagrass (*Cynodon dactylon*). *Weed Sci.* 31:385–390.
- Johnson, B. J. 1994. Biological control of annual bluegrass with *Xanthomonas campestris* pv. *Poannua* in bermudagrass. *HortScience.* 29:659–662.
- Johnson, B. J. and R. N. Carrow. 1995. Response of creeping bentgrass to iron applied in combination with herbicide-flurprimidol. *J. Turfgrass Manag.* 1:25–34.

- Johnson, B. J. and T. R. Murphy. 1995. Effect of paclobutrazol and flurprimidol on suppression of *Poa annua* spp *reptans* in creeping bentgrass (*Agrostis stolonifera*) greens. Weed Technol. 9:182–186.
- Johnson, B. J. and T. R. Murphy. 1996. Suppression of a perennial subspecies of annual bluegrass (*Poa annua* spp *reptans*) in a creeping bentgrass (*Agrostis stolonifera*) green with plant growth regulators. Weed Technol. 10:705–709.
- Jordan, D. L., A. C. York, and F. T. Corbain. 1989. Effect of ammonium sulfate and bentazon on sethoxydim absorption. Weed Technol. 3:674–677.
- Juska, F. V. and A. A. Hanson. 1969. Nutritional requirements of *Poa annua* L. Agron. J. 61:466–468.
- Kaminski, J. E. and P. H. Dernoeden. 2007. Seasonal *Poa annua* L. emergence patterns in Maryland. Crop Sci. 47:775–781.
- Kells, J. J., C. E. Reick, R. L. Blevins, and W. M. Muir. 1980. Atrazine dissipation as affected by surface pH and tillage. Weed Sci. 28:101–104.
- Kelly, S.T., E. Coats, and D.S. Luthe. 1999. Mode of resistance of triazine-resistant annual bluegrass (*Poa annua*). Weed Technol. 13: 747–752.
- Keskin, M., Y. J. Han, R. B. Dodd, and A. Khalilian. 2008. Reflectance-based sensor to predict visual quality ratings of turfgrass plots. Appl. Eng. Agric. 24:855–860.
- Kleier, D. A. 1988. Phloem mobility of xenobiotics I. Mathematical model unifying the weak acid and intermediate permeability theories. Plant Physiol. 86:803–810.
- Kohler, E. A. and B. E. Branham. 2002. Site of uptake, absorption, translocations, and metabolism of ethofumesate in three turfgrass species. Weed Sci. 50:576–580.

- Koo, S. J., K. H. Hwang, M. S. Jeon, S. H. Kim, J. Lim, D. G. Lee, K. H. Chung, Y. K. Ko, J.W. Ryu, D. W. Koo, and J. C. Woo. 2010. Development of the new turf herbicide methiozolin. *Kor. J. Weed Sci.* 30: 323–329.
- Koo, S. J., K. H. Hwang, M. S. Jeon, S. H. Kim, J. Lim, D. G. Lee, N.G. Cho. 2014. Methiozolin [5-(2,6-Difluorobenzyl)oxymethyl-5-methyl-3-(3-methylthiophen-2-yl)-1,2-isoxazoline], a new annual bluegrass (*Poa annua* L.) herbicide for turfgrasses. *Pest Manag. Sci.* 70:156–162.
- Koskinen, W. C. and H. H.Cheng. 1983. Effects of experimental variables on 2,4,5-T adsorption-desorption in soil. *J. Environ. Qual.* 12: 325–330.
- Kuo, S. 1993. Calcium and phosphorus influence creeping bentgrass and annual bluegrass growth in acidic soils. *HortScience.* 28:713–716.
- La Mantia, J. M. and D. R. Huff. 2011. Instability of the greens-type phenotype in *Poa annua* L. *Crop Sci.* 51: 1784–1792.
- Ladlie, J. S., W. F. Meggitt, and D. Penner. 1976a. Effect of soil pH on microbial degradation, adsorption, and mobility of metribuzin. *Weed Sci.* 24:477–481.
- Ladlie, J. S., W. F. Meggitt, and D. Penner. 1976b. Effect of pH on metribuzin activity in the soil. *Weed Sci.* 24:505–507.
- Lee, J. N., S. J. Koo, K. H. Hwang, I. T. Hwang, D. J. Jeon, and H. R. Kim. 2007. Mode of action of a new isoxazoline compound. *Proc. 21<sup>st</sup> Asian Pacific Weed Sci. Conf.* Pp. 591–601.
- LeStrange, M., P. M. Geisel, D. W. Cudney, C. L. Elmore, and V. A. Gibeault. 2012. Annual bluegrass integrated pest management for home gardeners and landscape

- professionals. University of California Statewide Integrated Pest Management Program. Pub. No. 7464. 4p.
- Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. SAS® for Mixed Models. 2nd ed. Cary, NC: SAS Institute Inc. Pp.15–56.
- Lush, W. M. 1989. Adaptation and differentiation of golf course populations of annual bluegrass (*Poa annua*). *Weed Sci.* 37:54–59.
- Lycan, D. W. and S. E. Hart. 2006. Seasonal effects on annual bluegrass (*Poa annua*) control in creeping bentgrass with bispyribac-sodium. *Weed Technol.* 20: 722–727.
- Maschhoff, J. R., S. E. Hart, and J. L. Baldwin. 2000. Effect of ammonium sulfate on the efficacy, absorption, and translocation of glufosinate. *Weed Sci.* 48:2–6.
- Massey, J. H., J. M. Taylor, N. B. K. Chambers, G. E. Coats, and W. P. Henry. 2006. Iron antagonism of MSMA herbicide applied to bermudagrass: characterization of the Fe<sup>2+</sup>-MAA complexation reaction. *Weed Sci.* 54:23–30.
- McCarty, L. B. 2005. *Best Golf Course Management Practices*. 2nd ed. Upper Saddle River, NJ: Pearson Prentice Hall. Pp. 3–58.
- McCullough, P. E. and D. Gómez de Barreda. 2012. Cool-season turfgrass reseeding intervals for methiozolin. *Weed Technol.* 26:789–792.
- McCullough, P. E., Gómez de Barreda, D., and J. Yu. 2013. Selectivity of methiozolin for annual bluegrass control in creeping bentgrass as influenced by temperature and application timing. *Weed Sci.* 61:209–216.
- McCullough, P. E., S. E. Hart, D. Weisenberger, and Z. J. Reicher. 2010. Amicarbazone efficacy on annual bluegrass and safety on cool-season turfgrasses. *Weed Technol.* 24:461–470.

- McCurdy, J. D., J. S. McElroy, and G. K. Breeden. 2009. Yellow nutsedge (*Cyperus esculentus*) and large crabgrass (*Digitaria sanguinalis*) response to soil- and foliar-applied mesotrione. *Weed Technol.* 23:62–66.
- McDonald, S. J., P. H. Dernodden, and J. E. Kaminski. 2006. Creeping bentgrass tolerance and annual bluegrass control with bispyribac-sodium tank-mixed with iron and nitrogen. *Appl. Turfgrass Sci.* doi: 10.1094/ATS-2006-0811-01-RS.
- McElroy, J. S., F. H. Yelverton, T.W. Gannon, and J. W. Wilcut. 2004a. Foliar vs. soil exposure of green kyllinga (*Kyllinga brevifolia*) and false-green kyllinga (*Kyllinga gracillima*) to postemergence treatments of CGA-362622, halosulfuron, imazaquin, and MSMA. *Weed Technol.* 18:145–151.
- McElroy, J. S., G. K. Breeden, and G. Wehtje. 2011. Evaluation of annual bluegrass control programs for bermudagrass turf overseeded with perennial ryegrass. *Weed Technol.* 25:58–63.
- McElroy, J.S., M.L. Flessner, Z. Wang, F. Dane, R.H. Walker, and G.R. Wehtje. 2013. A Trp574 to Leu amino acid substitution in the ALS gene of annual bluegrass associates with resistance to ALS-inhibiting herbicides. *Weed Sci.* 61:21–25.
- McElroy, J. S., R. H. Walker, and E. van Santen. 2002. Patterns of variation in *Poa annua* populations as revealed by canonical discriminant analysis of life history traits. *Crop Sci.* 42:513–517.
- McElroy, J. S., R. H. Walker, G. R. Wehtje, and E. van Santen. 2004b. Annual bluegrass (*Poa annua*) populations exhibit variation in germination response to temperature, photoperiod, and fenarimol. *Weed Sci.* 52:47–52.



- McGlamery, M. D. and F. W. Slife. 1966. The adsorption and desorption of atrazine as affected by pH, temperature and concentration. *Weeds* 14:237–239.
- McNulty, M.S. and Askew, S. D. 2011. Effects of methiozolin rates on creeping bentgrass (*Agrostis stolonifera* L.) putting green turf. *Proc. South Weed Sci. Soc.* 64:294.
- Mitich, L. W. 1998. Annual Bluegrass (*Poa annua* L.). *Weed Technol.* 12:414–416.
- Mueller, T. C. and P. A. Banks. 1991. Flurtamone adsorption and mobility in three Georgia soils. *Weed Sci.* 39:275–279.
- Myers, D. F., R. Hanrahan, J. Michel, B. Monke, L. Mudge, L. Norton, C. Olsen, A. Parker, J. Smith, and D. Spak. 2009. Indaziflam/BCS-AA170717-- a new herbicide for preemergent control of grasses and broadleaves in turf and ornamentals. *Proc. South. Weed. Sci. Soc.* 62:394.
- Nam, J. H., K. Hwang, S. Koo, C. Kim, C. Cho, and Y. K. Ko. 2012. Synthesis and herbicidal activities of enantiopure methiozolins. *Bull. Korean Chem. Soc.* 33:297–300.
- Nearpass, D. C. 1965. Effects of soil acidity on the adsorption, penetration, and persistence of simazine. *Weeds* 13:341–346.
- Norsworthy, J. K., D. B. Johnson, G. M. Griffith, C. Starkey, M. J. Wilson, and J. Devore. 2011. Efficacy and tolerance of dry-seeded rice to methiozolin. *Proc. South Weed Sci. Soc.* 64:31.
- Norsworthy, J. K., S. M. Ward, D. R. Shaw, R. S. Llewellyn, R. L. Nichols, T. M. Webster, K. W. Bradley, G. Frisvold, S. B. Powles, N. R. Burgos, W. W. Witt, and M. Barrett. 2012. Reducing the risks of herbicide resistance: best management practices and recommendations. *Weed Sci. Special Issue*:31–62.

- Obrigawitch, T., F. M. Hons, J. R. Abernathy, and J. R. Gipxon. 1981. Adsorption, desorption, and mobility of metholachlor in soils. *Weed Sci.* 29:332–336.
- Ogle, R. E. and G. F. Warren. 1954. Fate and activity of herbicides in soils. *Weeds* 3:257–273.
- Olech, M. 1996. Human impact on terrestrial ecosystems in West Antarctica. *Proc. NIPR Symp. Polar Biol.* 9:299–306.
- Oliveira, R. S., D. G. Alonso, and W. C. Koskinen. 2011. Sorption-desorption of aminocyclopyrachlor in selected Brazilian soils. *J. Ag. and Food Chem.* 59:4045–4050.
- Osgerber, J.M. 1974. Processes affecting herbicide action in soil. *Pestic. Sci.* 4:247–258.
- O’Sullivan, P. A., J. T. O’Donovan, and W. M. Hamman. 1981. Influence of non-ionic surfactants, ammonium sulphate, water quality, and spray volume on the phytotoxicity of glyphosate. *Can. J. Plant Sci.* 61:391–400.
- Owen, M. D. K. 2008. Review Weed species shifts in glyphosate-resistant crops. *Pest. Manag. Sci.* 64:377–387.
- Park, B. S. and P. J. Landschoot. 2003. Effect of dazomet on annual bluegrass emergence and creeping bentgrass establishment in turf maintained as a golf course fairway. *Crop Sci.* 43:1387–1394.
- Patterson, M. G., G. A. Buchanan, R. H. Walker, and R. M. Patterson. 1982. Fluometuron in soil solution as an indicator of its efficacy in three soils. *Weed Sci.* 30:688–691.
- Perry, D. H., J. S. McElroy, and R. H. Walker. 2009. The influence of amicarbazone on creeping bentgrass (*Agrostis stolonifera*) photochemical efficiency. *Proc. South. Weed. Sci. Soc.* 62:386.

- Perry, D. H., J. S. McElroy, M. C. Doroh, and R. H. Walker. 2011a. Indaziflam utilization for controlling problematic turfgrass weeds [Online behind pay wall]. *Appl. Turfgrass Sci.* 1–7. doi:10.1094/ATS-2011-0428-01-RS.
- Perry, D. H., J. S. McElroy, F. D. Dane, E. van Santen, and R. H. Walker. 2012. Triazine-resistant annual bluegrass (*Poa annua*) populations with Ser264 mutation are resistant to amicarbazone. *Weed Sci.* 60:355–359.
- Perry, D. H., J. S. McElroy, and R. H. Walker. 2011b. Effects of soil vs. foliar application of amicarbazone on annual bluegrass (*Poa annua*). *Weed Technol.* 25:604–608.
- Pierce, N. L., Eggens, J. L. and C. P. M. Wright. 1987. Mowing fairways with greensmowers. *Greenmaster* 2:7–11.
- Renner, K. A., W. F. Meggitt, and D. Penner. 1988. Effect of soil pH on imazaquin and imazethapyr adsorption to soil and phytotoxicity to corn (*Zea mays*). *Weed Sci.* 36:78–83.
- Roberts, H. A. and P. A. Feast. 1973. Emergence and longevity of seeds of annual weeds in cultivated and undisturbed soil. *J. Appl. Ecol.* 10:133–143.
- Rodriguez, I. R., L. B. McCarty, and J. K. Higginbottom. 2001. Postemergence annual bluegrass control in dormant common bermudagrass. *Intl. Turfgrass Soc.* 9:1041–1044.
- Roskamp, J. M., G. S. Chahal, and W. G. Johnson. 2013. The effects of cations and ammonium sulfate on the efficacy of dicamba and 2,4-D. *Weed Technol.* 27:72–77.
- Ross, M. A., and C. A Lembi. 1999. *Applied Weed Science*. 2<sup>nd</sup> ed. Upper Saddle River, NJ: Prentice-Hall, Inc. Pp. 126–143.

- Sakaliene, O., S. K. Paperinik, W. C. Koskinen, and K. A. Spokas. 2007. Sorption and predicted mobility of herbicides in Baltic Soils. *J. Environ. Sci. Health., Part B.* 42:641–647.
- Savage, K. E and R. D. Wauchope. 1974. Flumeturon adsorption-desorption equilibria in soil. *Weed Sci.* 22:106–110.
- Senseman, S. A., ed. 2007. *Herbicide Handbook*. 9th ed. Lawrence, KS: Weed Science Society of America. Pp. 14; 243–246; 275–278; 286–288; 298–301; 331–332.
- Shea, P. J. 1989. Role of humified organic matter in herbicide adsorption. *Weed Technol.* 3:190–197.
- Soil Survey Staff. 2004. R. Burt (ed.). *Soil survey laboratory methods manual*. Version 4.0. Soil survey investigations report No. 42. USDA-NRCS. GPO, Washington, DC.
- Soll J., G. Schultz, J. Joyard, R. Douce, and M. Block. 1985. Localization and synthesis of prenylquinones in isolated outer and inner envelope membranes from spinach chloroplasts. *Arch. Biochem. Biophys.* 238:290–299.
- Sprankle, P., W. F. Meggitt, and D. Penner. 1975. Adsorption, mobility, and microbial degradation of glyphosate in the soil. *Weed Sci.* 23:229–234.
- Stougaard, R. N., P. J. Shea, and A. R. Martin. 1990. Effect of soil type and pH on adsorption, mobility, and efficacy of imazaquin and imazethapyr. *Weed Sci.* 38:67-73.
- Strebe, T.A. and R. E. Talbert. 2001. Sorption and mobility of flumetsulam in several soils. *Weed Sci.* 49:806–813.
- Talbert, R. E. and O. H. Fletchall. 1965. The adsorption of some s-triazines in soils. *Weeds* 13:46-52.

- Tisdale, S. L., W. L. Nelson and J. D. Beaton. 1985. Soil Fertility and Fertilizers. 4<sup>th</sup> ed. New York: NY: Macmillian Publishing Company. Pp. 95–111.
- Toler, J. E., T. G. Willis, A. G. Estes, and L. B. McCarty. 2007. Postemergence annual bluegrass control in dormant nonoverseeded bermudagrass turf. *HortSci.* 42:670–672.
- Trappe, J. M., A. J. Patton, D. Weisenberger, G. Breeden, and J. Brosnan. 2012. Methiozolin rate and spring application timing affect annual bluegrass control on putting greens. *Proc. Crop Sci. Soc. of Amer.* 102-11.
- Tutin, T. G. 1957. Biology and Control of Eight *Poa annua* L. Ecotypes. M.S. thesis. Auburn AL: Auburn University, Auburn, AL. 72 p.
- Upchurch, R. P. and D. D. Mason. 1962. The influence of soil organic matter on the phytotoxicity of herbicides. *Weeds* 10:9–14.
- USGA Green Section. 2004. USGA recommendations for a method of putting green construction. [http://www.usga.org/course\\_care/articles/construction/greens/USGA-Recommendations-For-A-Method-Of-Putting-Green-Construction\(2\)/](http://www.usga.org/course_care/articles/construction/greens/USGA-Recommendations-For-A-Method-Of-Putting-Green-Construction(2)/) Accessed: December 31, 2013.
- Varco, J. J. and J. B. Sartain. 1966. Effects of phosphorous, sulfur, calcium hydroxide, and pH on growth of annual bluegrass. *Soil Sci. Soc. Am. J.* 50:128–132.
- Waddington, D. V., T. R. Turner, J. M. Duich, and E. L. Moberg. 1978. Effect of fertilization on Pennncross creeping bentgrass. *Agron. J.* 70:713–718.
- Walton, D. W. H. and R. I. L. Smith. 1973. Status of the alien vascular flora of South Georgia. *36:79–97.*

- Warwick, S. I. 1979. The biology of Canadian weeds. 37. *Poa annua* L. Can. J. Plant Sci. 59:1053–1066.
- Wauchope, R. D, S. Yeh, J. B. H. J. Linders, R. Kloskowski, K. Tanaka, B. Rubin, A. Katayama, W. Kördel. Z. Gerstl, M. Lane, and J. B. Unsworth. 2002. Review pesticide soil sorption parameters: theory, measurement, uses, limitations and reliability. Pest. Manag. Sci. 58:419–445.
- Wauchope, R. D, T. M. Buttler, A. G. Hornsby, P. W. M. Augustijn-Beckers, and J. P. Burt. 1992. The SCS/ARS/CES pesticide properties database for environmental decision-making. Rev. Environ. Contam. Toxicol. 123:1–156.
- Weber, J. B. 1995. Physiochemical and mobility studies with pesticides. Pages 99–115 in M. L. Leng, E. M. K. Leovey, and P. L. Zubkoff, eds. Agrochemical Environmental Fate: State of the Art. Boca Raton, FL: CRC Press.
- Weber, J. B., S. B. Weed, and T. M. Ward. 1969. Adsorption of s-triazines by soil organic matter. Weed Sci. 17:417–421.
- Webster, T. M. 2004. Weed Survey—Southern States. Grass crop subsection. Proc., South. Weed Sci. Soc. 57:404–426.
- Wehtje, G. R. and R. H. Walker. 2002. Response of two annual bluegrass varieties to preemergence and postemergence rimsulfuron. Weed Technol. 16:612–616.
- Wehtje, G. R., C. H. Gilliam, M. E. Miller, and J. E. Altland. 2006. Foliar vs. root sensitivity of hairy bittercress (*Cardamine hirsuta*) to isoxaben. Weed Technol. 20:326–333.
- Wehtje, G. R., M. E. Miller, T. L. Grey, and W. R. Barwner. 2007. Comparison between x-ray film- and phosphorescence imaging-based autoradiogram for the visualization of herbicide translocation. Weed. Technol. 21:1109–1114.

- Wehtje, G. R., R. Dickens, J. W. Wilcut, and B. F. Hajek. 1987. Sorption and mobility of sulfometuron and imazapyr in five Alabama soils. *Weed Sci.* 35:858–864.
- Wehtje, G. R., R. H. Walker, and J. N. Shaw. 2000. Pesticide retention by inorganic soil amendments. *Weed Sci.* 48:248–254.
- Wells, G. J. 1974. The biology of *Poa annua* and its significance in grassland. *Herbage Abstr.* 44:385–391.
- Wilcut, J. W., G. R. Wehtje, M. G. Patterson, T. A. Cole, and T. V. Hicks. 1989. Absorption, translocation, and metabolism of foliar-applied chlorimuron in soybeans (*Glycine max*), peanuts (*Arachis hypogaea*), and selected weeds. *Weed Sci.* 37:175–180.
- Williams, W., G. Wehtje, and R. H. Walker. 2003. CGA-362622: Soil behavior and foliar versus root absorption by torpedograss (*Panicum repens*). *Weed Technol.* 17:366–372.
- Woosley, P. B., D. W. Williams, and A. J. Powell. 2003. Postemergence control of annual bluegrass (*Poa annua* spp. *reptans*) in creeping bentgrass (*Agrostis stolonifera*) turf. *Weed Technol.* 17:770–776.
- Xu, X. and C. F. Mancino. 2001. Zinc requirements of annual bluegrass and creeping bentgrass. *HortScience.* 36:784–786.
- Xue, S. K. and H. M. Selim. 1995. Modeling adsorption-desorption kinetics in alachlor in a Typic Fradiudalf. *J. Environ. Qual.* 24:896–903.
- Yelverton, F. H. and L. B. McCarty. 2001. Tolerance of perennial ryegrass and *Poa annua* control with herbicides in overseeded bermudagrass. *Int. Turfgrass Soc. Res. J.* 9:1050–1055.
- Yeomans, J.C. and J.M. Bremner. 1991. Carbon and Nitrogen Analysis of Soils by Automated Combustion. *Commun. Soil Sci. Plant Anal.* 22:843–850.

Younger, V. B. 1959. Ecological studies on *Poa annua* in turfgrass. J. Br. Grassl. Soc. 14:233–237.

Yu, J. and P. E. McCullough. 2014. Methiozolin efficacy, absorption, and fate in six cool-season grasses. Crop Sci. 54:1211–1219.