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

Michael L. Flessner, Glenn R. Wehtje, and J. Scott McElroy*

Annual bluegrass is a problematic turfgrass weed. 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 PRE-applied methiozolin as influenced by rate and soil type and from 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 and 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. Because control is limited from a single methiozolin application (as observed in POST experiments), successful field application of methiozolin requires multiple and timely applications directed toward the roots and/or foliage of annual bluegrass.

Nomenclature: Methiozolin, MRC-01, 5-(2,6-difluoro-benzyloxymethyl)-5-methyl-3-(3-methyl-thiophen-2-yl)-4,5-dihydro-isoxazole; annual bluegrass, *Poa annua* L.

Key words: Foliar absorption, radio-labeled herbicides, root absorption, soil-plus-foliar.

Annual bluegrass is a problematic turfgrass weed because of its prolific seedhead production, bunch-type growth habit, color, and other characteristics (Beard 1973). Annual bluegrass is especially troublesome in golf course putting greens because of 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 1995; Johnson and Murphy 1996). These management strategies can successfully shift the competitive advantage away from annual bluegrass and 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 short-lived perennial, making herbicidal PRE control problematic (Callahan and McDonald 1992; Gibeault and Goetze 1972; McElroy et al. 2004a). Additionally, herbicide-resistant strains exist to many modes-of-action, which may interfere with PRE and POST control (Anonymous 2012; Heap 1997; Isgrigg et al. 2002; 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,b, 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.) Beauv.], goosegrass [*Eleusine indica* (L) Gaertn.], and annual bluegrass (Brosnan et al. 2013; 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 and timely applications (Haguewood et al. 2012; Han and Kaminski 2011, 2012; Hoyle et al. 2012; Hwang and Koo 2009; 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 broadleaf 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 the absorption and to a lesser extent the 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 because of low water solubility. Overall, herbicides with $\log K_{ow}$ values between 2.5 and 3.5 generally exhibit good root to shoot translocation (Briggs et al. 1982; Hsu et al 1990). Phloem mobility is optimal at $\log K_{ow}$ values of 0 to 1.0 (Briggs et al. 1982; Hsu et al. 1990; Kleier 1988). Therefore methiozolin is expected to be absorbed by the roots and shoots with limited root to shoot translocation but little to no phloem mobility.

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Among herbicides that can selectively control annual bluegrass in turfgrass, control varies with herbicide placement. Brosnan et al. (2013) 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. 2013). Other researchers have noted annual bluegrass control varies with herbicide placement with other herbicides. Perry et al. (2011) 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. 2013; 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 because of 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 h 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, which had an 80 cm^2 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^{-1}); 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. LS means 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).

PRE Experiments. Experiments were conducted to determine if methiozolin (MRC-01, Mughu Research Center, Daejeon, Korea) has PRE activity against germinating annual bluegrass seed. 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 v/v USGA grade sand to organic matter mix, pH 5.3). Methiozolin was applied at 1.68 and 3.36 kg ai ha^{-1}. A nontreated check was also included with each soil type. All herbicide-containing treatments contained nonionic surfactant at 0.25%v/v (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^{-1}. Above ground biomass was harvested 25 d 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.

POST 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 methiozolin rates previously described, two annual bluegrass growth stages ('small,' approximately two tillers and 'large,' approximately six 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. 2004b; McCurdy et al. 2009; Wehtje and Walker 2002; Williams et al. 2003).

Above ground biomass was harvested 25 DAT in the same manner as PRE experiments. Additionally in POST experiments, above ground 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

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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^{-1} application rate in 187 L ha^{-1} carrier volume. Treatment solution was prepared by combining ^{14}C-methiozolin and nonlabeled methiozolin such that the total concentration was 11.98 mg ml^{-1} and the radioactive concentration was 0.084 MBq $ml-^{1}$. A single ~5µL 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 h 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 ≥ 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}C-labeled and nonlabeled methiozolin such that the total concentration and radioactive concentration was 5.0 µg ml^{-1} (ppm) and 415.0 MBq mL^{-1}, respectively. The 5.0 ppm concentration was selected since it represents a 2.24 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}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

PRE Experiments. Methiozolin resulted in near complete annual bluegrass control [99.9%; 95% confidence interval (CI95) 94 to 106%]. Control was not affected by either soil type or methiozolin rate ($P = 0.994$ and $P = 0.999$, respectively), indicating that the only significant factor was whether methiozolin was applied or not. These data indicate that methiozolin at 1.68 kg ha^{-1} 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.

POST Selective Placement Experiments. Annual bluegrass control 25 DAT: POST selective placement controlled annual bluegrass ≤ 50% (data not shown). Growth stage was the only significant parameter, indicating that herbicide rate, soil-type, and treatment placement did not influence annual bluegrass control. Methiozolin resulted in better control of the small growth stage compared to the large growth stage. Methiozolin controlled the small growth stage 50% (CI95 44 to 56%) and the large growth stage 28% (CI95 24 to 34%).

Annual bluegrass control 39 DAT was generally greater than 25 DAT, but maximum control remained < 80% (Table 1). 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

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Table 1. POST annual bluegrass control from methiozolin as influenced by rate, growth stage, and treatment placement.[a]

Treatment					95% confidence limits	
Rate	Growth stage	Placement	Control[b]		Lower	Upper
kg ha^{-1}					%	
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

[a] Evaluated 39 days after treatment; data pooled over soil types.
[b] Control measured as percent reduction in above ground 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.

likely because of 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. (2013) also reported similar annual bluegrass control between soil-based (silt loam) and sand-based rootzones.

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 kg ai ha^{-1}) was 61% (CI95 54 to 67%) while control from the low rate (1.68 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 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 2- and 4-leaf stage annual bluegrass from methiozolin applied at 0.50 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 1). 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 1). Previous research reported that soil-only– and foliar-plus-soil–applied methiozolin (1.0 kg ha^{-1}) treatment placement resulted in greater annual bluegrass control compared to foliar-only treatment placement (Brosnan et al. 2013). Current indications are that annual bluegrass control in the field requires multiple applications and complete control may still not be achieved (Brosnan et al. 2013; 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.

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 2 to show trends. This indicates that foliar methiozolin absorption and translocation is likely complete 24 h 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 unlikely that methiozolin translocates in the phloem, which is also consistent with

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Table 2. Foliar absorption and translocation of ^{14}C-methiozolin by annual bluegrass over time.[a]

Sample	Hours after treatment[b]			Mean[c]		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

[a] Leaf spotting was a 5 µL droplet of methiozolin corresponding to a 2.24 kg ha^{-1} rate.
[b] Time after treatment not significant in ANOVA (P = 0.122) but presented to show trends in data.
[c] Means sharing a letter are not significantly different according to the adjusted 95% confidence interval.

its logK_{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 because of minimal translocation to the remainder of the plant.

Autoradiograms are in general agreement with data obtained through ^{14}C-methiozolin quantification (Figure 1). 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}C found in the different tissues ($P < 0.001$) and the amount of total ^{14}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 was < 8 µg methiozolin per g plant tissue (Table 3). Methiozolin translocation to the crown was ≤ 30 µg methiozolin per g plant tissue, indicating that methiozolin is absorbed by the roots and is translocated to the crown of annual bluegrass. This absorption and translocation pattern is consistent with the logK_{ow} value for methiozolin (3.9). Herbicides with logK_{ow} values in this range have excellent absorption because of their membrane permeation, but are not well translocated in the xylem because of 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 because of 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}C-methiozolin quantification (Figure 1). 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 logK_{ow} value for methiozolin (3.9), which suggests excellent root absorption, but difficulty in translocation because of poor partitioning into the xylem sap (Briggs et al. 1982; Hsu et al. 1990). As with foliage treatment, the autoradiogram of ^{14}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. 2013).

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 acropetally, presumably through the xylem. While current data are limited, a successful methiozolin application in the field likely requires both root and foliar exposure, because of limited translocation. Because 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.

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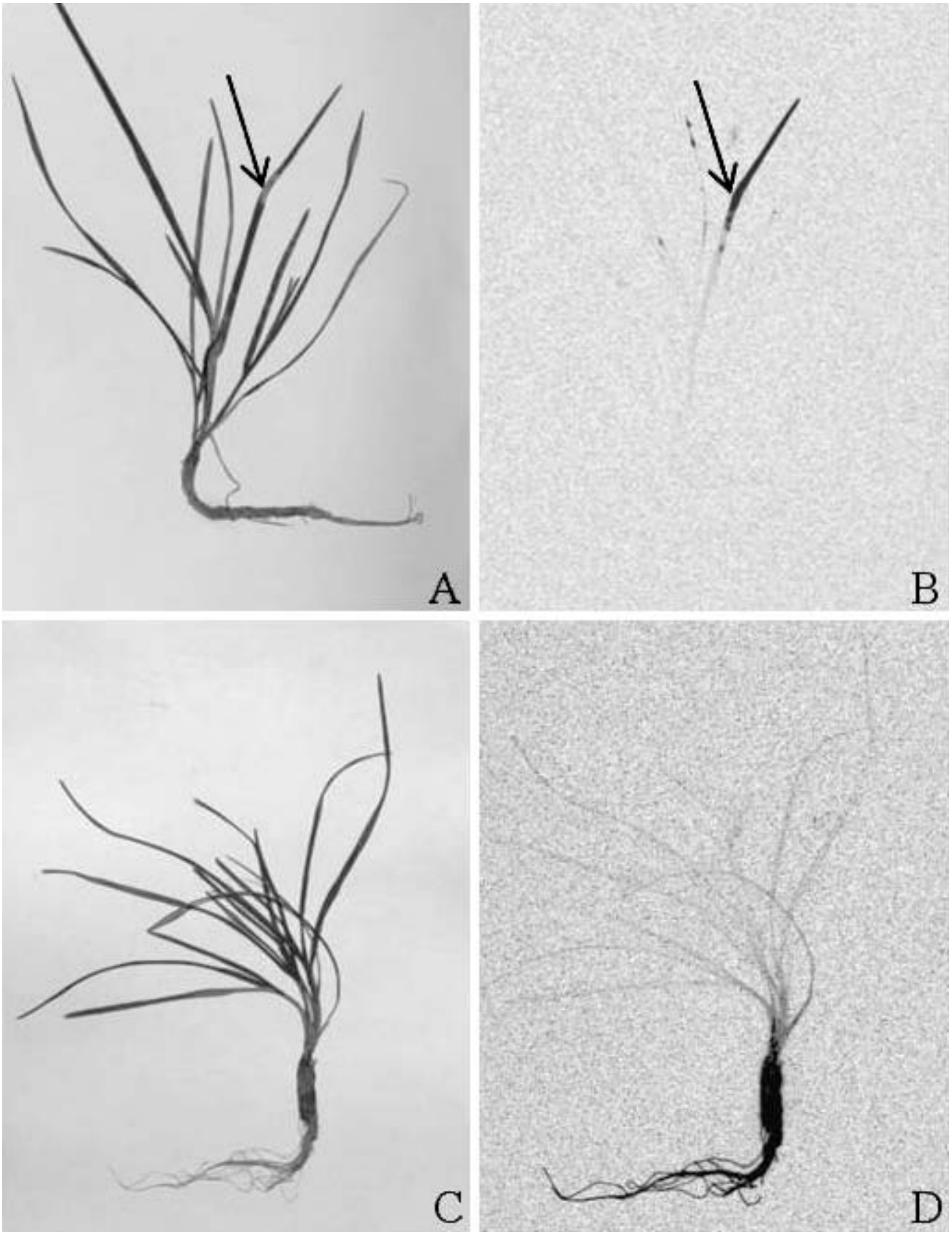

Figure 1. Annual bluegrass after treatment with ^{14}C-methiozolin. Rate simulated was 2.24 kg ha^{-1} for foliar and root application. (A) Plant which received foliar application. Arrow indicates leaf spotting location. (B) Autoradiogram of A, darkness indicates greater abundance of ^{14}C-methiozolin.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

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Table 3. Root absorption and translocation of ^{14}C-methiozolin by annual bluegrass over time.[a]

Time after treatment[b]	Tissue sample	Mean[c]		95% confidence limits Lower	Upper
h		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

[a] Solution contained 5 ppm methiozolin corresponding to a 2.24 kg ha^{-1} rate.
[b] Contrast statements did not detect differences between 48 and 72 h after treatment (P = 0.649).
[c] Means sharing a letter within time after treatment are not significantly different according to the adjusted 95% confidence interval.

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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.

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. 2012. Herbicide resistant annual bluegrass globally. http://www.weedscience.org/In.asp. Accessed June 5, 2012.

Beard, J. B. 1973. Turfgrass: Science and Culture. Englewood Cliffs, NJ: Prentice-Hall.

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.

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.

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. M. Henry, G. K. Breeden, T. Cooper, and T. J. Serensits. 2013. Methiozolin efficacy for annual bluegrass control on sand- and soil-based creeping bentgrass putting greens. Weed Technol 27:123–128.

Callahan, L. M. and E. R. McDonald. 1992. Effectiveness of bensulide in controlling two annual bluegrass (*Poa annua*) subspecies. Weed Technol. 6:97–103.

Dickens, R. 1979. Control of annual bluegrass (*Poa annua*) in overseeded bermudagrass (*Cynodon* spp.) golf greens. Weed Sci. 27:642–644.

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.

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. 127 p.

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. Amer. 105–27.

Han, K. M. and J. Kaminski. 2011. Control of perennial annual bluegrass biotypes with postemergence herbicides. Proc. Crop Sci. Soc. Amer. 104–30.

Han, K. M. and J. Kaminski. 2012. Influence of methiozolin rates and application timings on *Poa annua* populations. Proc. Crop Sci. Soc. Amer. 366–25.

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.

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. 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.

Hwang, K. and S. Koo. 2009. Herbicidal activity of MRC-01 on *Poa annua*. Proc. South Weed Sci. Soc. 62:194.

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.

Isgrigg, J., F. H. Yelverton, C. Brownie, and L. S. Warren. 2002. Dinitroaniline resistant annual bluegrass in North Carolina. Weed Sci. 50:86–90.

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 stonolifera*) green with plant growth regulators. Weed Technol. 10:705–709.

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.

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. Pages 591–601 *in* Proc. 21st Asian Pacific Weed Sci. Conf.

Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. Pages 15–56 *in* SAS® for Mixed Models. 2nd ed. Cary, NC: SAS Institute Inc.

Lycan, D. W. and S. E. Hart. 2006. Foliar and root absorption and translocation of bispyribac-sodium in cool-season turfgrass. Weed. Technol. 20:1015–1022.

McCullough, P. E. and D. Gómez de Barreda. 2012. Cool-season turfgrass reseeding intervals for methiozolin. Weed Technol. 26:789–792.

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.

McElroy, J. S., M. L. Flessner, Z. Wang, F. Dane, R. H. Walker, and G. R. Wehtje. 2012. A Trp574 to Leu amino acid substitution in the ALS gene of annual bluegrass (*Poa annua*) is associated with resistance to ALS-inhibiting herbicides. Weed Sci. 61:21–25.

McElroy, J. S., R. H. Walker, G. R. Wehtje, and E. van Santen. 2004a. Annual bluegrass (*Poa annua*) populations exhibit variation in germination response to temperature, photoperiod, and fenarimol. Weed. Sci. 52:47–52.

McElroy, J. S., F. H. Yelverton, T. W. Gannon, and J. W. Wilcut. 2004b. 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.

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.

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.

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.

Perry, D. H., J. S. McElroy, and R. H. Walker. 2011. Effects of soil vs. foliar application of amicarbazone on annual bluegrass (*Poa annua*). Weed Technol. 25:604–608.
Senseman, S. A., ed. 2007, Pages 14; 298–301 *in* Herbicide Handbook. 9th ed. Lawrence, KS: Weed Science Society of America.
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.
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. G., 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. and R. H. Walker. 2002. Response of two annual bluegrass varieties to preemergence and postemergence rimsulfuron. Weed Technol. 16:612–616.
Wehtje, G. R., R. H. Walker, and J. N. Shaw. 2000. Pesticide retention by inorganic soil amendments. Weed Sci. 48:248–254.
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.

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