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Selectivity of Methiozolin for Annual Bluegrass (*Poa annua*) Control in Creeping Bentgrass as Influenced by Temperature and Application Timing

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Methiozolin controls annual bluegrass in creeping bentgrass but application timing and temperature could influence efficacy in turf. In field experiments, sequential methiozolin applications totaling 3.36 kg ai ha^{-1} provided excellent (> 90%) annual bluegrass control at 8 wk after initial treatment when treatments were initiated in February/March or May but programs totaling 0.84 and 1.68 kg ha^{-1} provided poor control (< 70%) at both timings. Methiozolin at all rates caused minimal turf injury (< 8%) but creeping bentgrass was only injured from February/March applications. In growth chamber experiments, creeping bentgrass injury from methiozolin at 10 C was 2 and 4 times greater than at 20 C and 30 C, respectively, while annual bluegrass injury was similar across temperatures. In laboratory experiments, annual bluegrass had more foliar absorption of ^{14}C-methiozolin than creeping bentgrass at 30/25 C (day/night), compared to 15/10 C, but translocation was similar at both temperatures as > 90% of absorbed ^{14}C remained in the treated leaf after 72 h. Annual bluegrass distributed and recovered more radioactivity to shoots from root-applied ^{14}C-methiozolin than creeping bentgrass while both species had about 2 times more distribution to shoots at 30/25 C than 15/10 C. Metabolites were not detected in annual bluegrass or creeping bentgrass at 1, 3, or 7 d after treatment when grown at 15/10 C or 30/25 C suggesting uptake and translocation contributes to methiozolin selectivity in turfgrass.

Nomenclature: Annual bluegrass, *Poa annua* L.; creeping bentgrass, *Agrostis stolonifera* L.
Key words: Metabolism, temperature, translocation, turfgrass, uptake.

Annual bluegrass is a problematic weed in creeping bentgrass golf greens. Compared to creeping bentgrass, annual bluegrass has a lighter green color, coarser leaf texture, and produces unsightly seedheads that reduce turf quality and ball roll distances (Sprague and Burton 1937). Annual bluegrass is undesirable in polyculture with creeping bentgrass because of poor disease, drought, and wear tolerances that require more intensive management for successful culture (Lush 1989; Sprague and Burton 1937). PRE herbicides labeled for creeping bentgrass golf greens often provide erratic annual bluegrass control because of poor efficacy or the presence of perennial biotypes not controlled from applications (Callahan and McDonald 1992; Juska and Hanson 1967).

Turf managers have limited herbicides registered for selective POST annual bluegrass control in creeping bentgrass golf greens. Amicarbazone is a triazolinone herbicide that effectively controls annual bluegrass in cool-season turfgrasses from 0.1 to 0.2 kg ai ha^{-1} (Anonymous 2012; McCullough et al. 2010). The current label does not restrict amicarbazone use on golf greens but single application must not exceed 0.05 kg ha^{-1} because of creeping bentgrass injury (Anonymous 2012). Bispyribac-sodium is an acetolactate synthase inhibitor that may be used from 74 to 111 g ai ha^{-1} and sequential applications in spring can selectively control annual bluegrass in creeping bentgrass fairways (Lycan and Hart 2006a). However, applications to golf greens are often injurious and bispyribac-sodium use is restricted to creeping bentgrass fairways (Anonymous 2007; McCullough and Hart 2009; Teuton et al. 2007). Ethofumesate is labeled for POST applications in creeping bentgrass but rates must be reduced for other turf species, such as tall fescue [*Lolium arundinaceum* (Schreb.) S.J. Darbyshire], which reduces efficacy for annual bluegrass control (Johnson et al. 1989). Plant growth regulators, flurprimidol and paclobutrazol, are used to suppress annual bluegrass populations in creeping bentgrass golf greens but treatments must be applied in long-term sequential programs and often do not completely eliminate infestations (Johnson and Murphy 1995, 1996; Woosley et al. 2003).

A limitation to the efficacy of POST herbicides for annual bluegrass control is seasonal application timing. For example, bispyribac-sodium must be applied in spring when temperatures range 25 to 30 C to maximize annual bluegrass control and creeping bentgrass safety (Lycan and Hart 2006a; McCullough and Hart 2006). Amicarbazone should be applied at temperatures ranging 20 to 25 C as practitioners may lose selectivity in creeping bentgrass when temperatures exceed this range (Anonymous 2012; McCullough et al. 2010). Applications of amicarbazone and bispyribac-sodium in fall have shown to cause excessive injury to creeping bentgrass compared to spring which further limits seasonal application timings in most areas of the U.S. (Lycan and Hart 2006a; McCullough et al. 2010). Ethofumesate provides erratic levels of POST annual bluegrass control and must be used in fall because of reduced efficacy on mature annual bluegrass in spring (Johnson 1983). Resistance of annual bluegrass populations to these modes of action have been reported which may further exacerbate limitations for effective applications (Kelly et al. 1999; Perry et al. 2012).

Methiozolin is a POST herbicide currently under evaluation for selective annual bluegrass control in creeping bentgrass. Methiozolin is an isoxazoline herbicide that is believed to disrupt cell wall biosynthesis in susceptible species (Lee et al. 2007). In preliminary experiments, sequential applications of methiozolin safely controlled annual bluegrass in creeping bentgrass golf greens without reducing putting green quality (P. McCullough, personal observation). Methiozolin could offer turf managers a new chemistry for selective annual control bluegrass in creeping bentgrass but the influence of application timing and temperature on efficacy has received limited investigation. The objective of this research was to evaluate the influence of temperature on (1)

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Table 1. Creeping bentgrass injury, annual bluegrass control, and turf quality following applications of methiozolin to golf greens in two combined field experiments, 2011–2012, in Marietta, GA and Peachtree City, GA.

Application Timing[b]	Treatment[a]		Creeping bentgrass injury			Annual bluegrass control		Turf quality
	Herbicide	Rate	2 WAIT[c]	4 WAIT	8 WAIT	4 WAIT	8 WAIT	8 WAIT
		kg ai ha^{-1}	%					1 to 9
Feb/March	amicarbazone	0.05 + 0.05 + 0.05	1	0	0	17	23	7.9
	methiozolin	0.42 + 0.42	1	0	0	14	23	7.9
		0.84 + 0.84	2	1	1	28	54	7.5
		1.68 + 1.68	6	7	1	43	92	6.9
May	amicarbazone	0.05 + 0.05 + 0.05	6	5	3	83	98	5.6
	methiozolin	0.42 + 0.42	0	0	0	35	50	7.3
		0.84 + 0.84	0	0	0	37	62	6.9
		1.68 + 1.68	0	0	1	45	93	5.9
		Nontreated	-	-	-	-	-	7.7
		$LSD_{0.05}$[d]	4	4	2	13	22	0.9
		Timing	NS	NS	NS	*	*	*
		Treatment	*	*	NS	*	*	*
		Timing by Treatment	*	*	NS	*	*	*

[a] Products applied were Xonerate (amicarbazone 70DF), Arysta LifeScience, 15401 Weston Parkway Suite 150, Cary, NC 27513; and methiozolin (25%EC), Moghu Corp., Kribb BVC 311, Yuseong, Daejeon 305-333, Korea. Nonioinic surfactant (Chem Nut 80-20, Chem Nut Inc., Albany, GA 31706) was included at 0.25% and 0.125% v/v of spray solution for amicarbazone and methiozolin, respectively.

[b] Applications were made in 2011 on February 16 and March 16 for methiozolin applications beginning in February/March timing and May 11 and June 13 for methiozolin applications beginning in May. In 2012, methiozolin was applied March 14 and April 11 for the February/March timing and May 23 and June 21 for the treatments beginning in May. Amicarbazone was applied on February 16, March 1, and March 16 for applications beginning in February/March timing and May 11, May 24, and June 13 for applications beginning in May. In 2012, amicarbazone applications were made on March 14, March 28, and April 11 for the February/March timing and May 23, June 7, and June 21 for the treatments beginning in May.

[c] WAIT = weeks after initial treatment.

[d] Means were separated with Fisher's Protected LSD test at the 0.05 significance level.

* Significant at 0.05 probability level. NS, not significant at 0.05 probability level.

methiozolin efficacy for annual bluegrass control in creeping bentgrass golf greens, (2) injury and growth responses of annual bluegrass and creeping bentgrass to methiozolin and (3) absorption, translocation, and metabolism of ^{14}C-methiozolin in annual bluegrass and creeping bentgrass.

Materials and Methods

Field Experiments. Experiments were conducted from February to July 2011 at the Marietta City Golf Course in Marietta, GA (33.94°N, 84.55°W) and from March to July 2012 at Flat Creek Golf Course in Peachtree City, GA (33.41°N, 84.58°W). In 2011, turf was a 'Penncross' creeping bentgrass golf green grown on a United States Golf Association specification soil with 2% organic matter and a 6.6 pH (USGA 1993). The green was overseeded with 'L-93' creeping bentgrass annually since 2006. In 2012, turf was a 'Crenshaw' creeping bentgrass golf green grown on a Cecil-Appling sandy loam with 2.6% organic matter and a 5.5 pH. At both golf courses, creeping bentgrass was irrigated as needed and mowed daily during active growth at 3 to 3.2 mm height with a reel-mower and clippings collected. Fungicides including azoxystrobin, chlorothalonil, and propiconazole were applied to control diseases and turf was fertilized to meet deficiencies from soil tests with nitrogen inputs ranging 98 to 175 kg N ha^{-1} yr^{-1}. Visual evaluations of annual bluegrass cover were made on a percent scale where 0 equaled no ground cover and 100 equaled complete plot coverage. From visual evaluations, the golf greens averaged 29% (± 2) and 48% (± 4) annual bluegrass cover on the day of initial treatments for the February/March timing in 2011 and 2012, respectively. Greens averaged 60% (± 6) and 40% (± 10) annual bluegrass cover on the day of initial treatments for the May application timing in 2011 and 2012, respectively. Annual bluegrass at both golf courses was a mixture of biotypes but was predominately perennial.

Experimental design was a randomized complete block with three and four replications of 0.9 × 3-m plots in 2011 and 2012, respectively. Methiozolin (25%EC, Moghu Corp., Yuseong, Daejeon 305-333, Korea) was applied at two initial application timings, including winter (delineated February/March) or late spring (delineated May), at 0, 0.42, 0.84, or 1.68 kg ai ha^{-1}. A sequential treatment was applied after 4 wk and all applications included a nonionic surfactant (Chem Nut 80-20. Chem Nut Inc., Albany, GA 31706) at 0.125% v/v as previously used in preliminary experiments. Amicarbazone (Xonerate 70DF, Arysta LifeScience, 15401 Weston Parkway Suite 150, Cary, NC 27513) was applied as a standard comparison at 0.05 kg ha^{-1} in three biweekly applications at both application timings. Amicarbazone rates were chosen based on label recommendations for creeping bentgrass (Anonymous 2012) and a nonionic surfactant was included at 0.25% v/v. Application dates are presented in Table 1. Treatments were applied by making three passes per plot with CO_2 pressured sprayers calibrated to deliver a total 561 L ha^{-1} at 255 kPa with a single 9504E flat-fan nozzle (Tee Jet, Spraying Systems Co., Roswell, GA 30075).

Annual bluegrass cover was visually rated on a percent scale where 0 equaled no cover and 100 equaled complete plot cover at 0 and 8 weeks after initial treatments (WAIT) for the two application timings. Annual bluegrass control was visually measured on a percent scale where 0 equaled no control and 100 equaled complete control at 4 and 8 WAIT. Creeping bentgrass injury was visually evaluated 2, 4, and 8 WAIT on a percent scale where 0 equaled no injury and 100% equaled completely dead turf. Turf quality was visually rated at 8 WAIT on a 1 to 9 scale where 1 equaled dead turf and 9 equaled dense, uniform turf. Data were subjected to analysis

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of variance and significance of main effects (herbicide and application timing) and interactions were determined at the 0.05 probability level. Means were separated with Fisher's Protected LSD test at $\alpha = 0.05$. Year by treatment interactions were not detected, and thus, results were pooled over years.

Growth Chamber Experiments. Two experiments were conducted at the University of Georgia in Griffin, GA from September to December 2011. Multi-tiller plants of 'Pen-cross' creeping bentgrass and indigenous annual bluegrass were collected from local fields and single plants were placed in plastic pots, 79-cm^2 by 9-cm depth, with a sandy loam soil with 2% organic matter and 6.0 pH. Grasses were watered to promote growth and clipped weekly with sheers at a 5-cm height with clippings returned. Once grasses resumed active growth in a greenhouse, pots were placed in growth chambers set for 10, 20, or 30 C with a 12-h photoperiod and approximately 80% relative humidity. Grasses were acclimated for 1 wk in growth chambers prior to treatments.

Methiozolin (25%EC) was applied at 0, 0.56, 1.12, 2.24, 3.36, 4.48, or 5.6 kg ai ha^{-1} with CO_2 pressured sprayers calibrated to deliver 374 L ha^{-1} with a single 9504E flat-fan nozzle (Tee Jet, Spraying Systems Co., Roswell, GA 30075). A second application was made after 4 wk. Visual plant quality was rated on a 0 to 10 scale where 0 equaled dead and 10 equaled ideal, healthy grass. Results were converted to percent of the untreated and delineated as "injury" for presentation. Clippings were harvested with sheers at 8 WAIT, oven dried at 50 C for 72 hr, and then weighed.

Data were subjected to analysis of variance and interactions of temperature and species were analyzed at the 0.05 probability level. Data were then subjected to regression analysis for a two-parameter exponential rise to peak and rates of methiozolin that caused 50% reductions in clipping yield (CR_{50}) from the untreated were determined at each temperature. Predicted rates that would injure annual bluegrass and creeping bentgrass 50% (I_{50}) and 20% (I_{20}) were calculated. These values were chosen because 50% would indicate significant herbicidal activity on annual bluegrass and 20% is considered unacceptable injury to fine turfgrass. Experiment by treatment interactions was not detected, and thus, results were pooled over the two experiments.

Foliar Absorption and Translocation. Two experiments were conducted in Griffin, GA in summer 2012. 'Penn A-4' creeping bentgrass and annual bluegrass were seeded in pots measuring 20.5-cm depth by 3.8-cm in diameter. Annual bluegrass was seeded with dried seedhead material collected through clippings of indigenous plants in Griffin, GA. Soil was an 80:20 (v:v) mixture of a coarse textured sand and peat moss. Pots were placed in a greenhouse set for 20/15 C (day/night) temperature with no supplemental lighting. Pots were irrigated to promote establishment and received two applications of a 28–7–14 fertilizer (Lesco MarcoN, 15885 Sprague Rd, Strongsville, OH 44136) at 24 kg N ha^{-1} prior to treatments.

Plants used for experiments were at a three to five tiller stage and selected based on uniformity of size and quality for both species. Containers were placed in a growth chamber (Percival Scientific, 505 Research Drive, Perry, IA 50220) set for 15/10 C or 30/25 C (day/night) with about 60% relative humidity and 12-h photoperiods of 350 µmol m^{-1} s^{-1}. Grasses were irrigated to prevent wilt and allowed to resume active growth in the growth chamber for 5 d before treatments. A broadcast application of methiozolin was made at 1.12 kg ha^{-1} at 374 L ha^{-1} with aforementioned CO_2 pressured sprayers outside of the growth chambers. Immediately after broadcast applications, two 1-µl droplets of ^{14}C-methiozolin [specific activity: 4.55 MBq mg^{-1}; labeled at isoxazoline ring carbon] were applied to the first fully expanded leaf with 167 Bq of radioactivity with a 5-µl microsyringe (Hamilton Co., Reno, NV 89502). Appropriate amount of formulated methiozolin was added to the spotting solution to simulate 1.12 kg ha^{-1} at 374 L ha^{-1}. A nonionic surfactant (Chem Nut 80-20, Chem Nut Inc., Albany, GA 31706) was added to the spotting solutions at 0.125% v/v to facilitate deposition of the droplet on leaves.

Plants (roots plus shoots) were harvested 4, 24, or 72 h after treatment (HAT). Treated leaves were excised from the base of the plant and rinsed with 5 ml of 20% methanol solution in a 20-ml scintillation vial. For the 24 and 72 HAT harvest, plants were separated into treated leaves, nontreated shoots, and roots. Samples were oven dried at 50 C for 72 h and then combusted in a biological oxidizer (Model OX-500, R.J. Harvey Instrument Corp., Hillsdale, NJ 076742). Radioactivity was then quantified with liquid scintillation spectroscopy (Model LS6500, Beckman-Coulter, Inc., Fullerton, CA 92834-3100) for 3 min per sample. Methodology of these experiments was similar to previous experiments evaluating uptake and translocation of radiolabeled herbicides in turfgrasses (Lycan and Hart 2006b).

Experimental design was completely randomized with four replications. Percent ^{14}C-methiozolin absorption was calculated by dividing the total radioactivity recovered in samples by the initial amount applied to the leaf. Percent ^{14}C distribution was determined by dividing the radioactivity recovered in plant parts by the total amount recovered per plant. Data were subjected to analysis of variance at the 0.05 probability level. Means were separated with Fisher's Protected LSD test at $\alpha = 0.05$.

Root Absorption and Translocation. Two experiments were conducted at the University of Georgia in Griffin, GA. Plants were seeded in aforementioned pots, soil, and greenhouse. After establishment, multi-tiller plants were collected based on size and uniformity of samples. Roots were washed free of soil and then grown hydroponically in half-strength Hoagland solution (Hoagland and Arnon 1950). Plants were placed through holes in a floating styrofoam board (Williams Foam, 12961 San Fernando Road, Sylmar, CA 91342) that facilitated root submergence in solution in a 4-L plastic tank. Sides of the tank were covered with aluminum foil to reduce light exposure to plant roots and an aquarium pump provided a constant supply of oxygen to the solution. The tank was then placed in a growth chamber (Conviron, 572 South Fifth Street, Pembina, ND 58271) set for 20 C with a 12-h photoperiod for 5 d.

Plants were then removed from the solution and placed individually in tubes measuring 5.1-cm^2 by 15-cm-depth with 25 ml of half-strength Hoagland solution spiked with 15.2 kBq L^{-1} of ^{14}C-methiozolin. Roots were submerged in the solution by placing cotton balls around the base of the plant and formulated methiozolin was added to simulate a

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1.12 kg ha^{-1} surface application rate. Tubes were placed in a growth chamber set for either 15/10 or 30/25 C (day/night temperatures) with 12-h photoperiods of approximately 400 μmol m^{-2} s^{-1}. Because of the lack of available chambers, one temperature regimen was evaluated at a time and settings were randomized per experimental run. Plants were harvested 48 HAT and roots were rinsed with 20% methanol solution to remove unabsorbed ^{14}C. Roots were then blotted with a paper towel and separated from shoots using sheers. Samples were oven-dried at 50 C for 72 hr, weighed, and combusted in a biological oxidizer (Model OX-500, R.J. Harvey Instrument Corp., Hillsdale, NJ 076742). Radioactivity was then quantified with liquid scintillation spectroscopy (Model LS6500, Beckman-Coulter, Inc., Fullerton, CA 92834-3100) for 3 min per sample.

Two separate experiments were conducted in completely randomized designs with four replications. Percent absorption was calculated by dividing the amount of radioactivity recovered in the whole plant by total radioactivity in the solution. Radioactivity recovery was calculated by dividing the total radioactivity per sample by dry weights in roots and shoots. Percent ^{14}C distribution was determined by dividing the radioactivity recovered in plant parts by the total amount recovered in the plant. Data were subjected to analysis of variance at the 0.05 probability level. Means were separated with Fisher's Protected LSD test at $\alpha = 0.05$. Interactions of species and temperature were not detected with experiments, and thus results were pooled over experiments.

Metabolism Experiments. Two experiments were conducted at the University of Georgia in Griffin, GA from January to April 2012. Single tillers of creeping bentgrass and annual bluegrass were transplanted from field samples and placed in aforementioned pots, soil, and greenhouses. Plants were allowed to develop four to five new tillers prior to treatments and were selected based on size and uniformity. Plants were placed in separate growth chambers (Conviron, Pembina, ND 58271) set for 15/10 or 30/25 C (day/night) with approximately 50% relative humidity and 12-hr-photoperiods of about 400 μmol m^{-2} s^{-1}. Grasses were irrigated to prevent wilt and allowed to resume active growth in the growth chamber for 72 h before treatments.

Experimental design was completely randomized with four replications. A broadcast application of methiozolin was made at 1.12 kg ha^{-1} at 374 L ha^{-1} with aforementioned CO_2 pressured sprayers. Immediately after broadcast applications, two 1-μl droplets of ^{14}C-methiozolin [specific activity: 4.55 MBq/mg; labeled at isoxazoline ring carbon] were applied containing 1.7 kBq of total radioactivity with a 5-μl microsyringe (Hamilton Co., Reno, NV 89502). Appropriate amount of formulated methiozolin was added to the spotting solution to simulate 1.12 kg ha^{-1} at 374 L ha^{-1}. A nonionic surfactant (Chem Nut 80-20, Chem Nut Inc., Albany, GA 31706) was added to the spotting solution at 0.125% v/v. Treated leaves were harvested 1, 3, or 7 d after treatments and rinsed with methanol solution, and stored in a freezer at −10 C until analysis.

Leaves were ground with liquid nitrogen in 1.5 ml tubes and filled with 0.75 ml of 70% acetone solution. Samples were then agitated with an automatic rotary shaker for 30 sec and placed in a sonication bath for 45 min. Samples were then centrifuged for 5 min, extract was placed in a separate tube, and the procedure was repeated with 0.75 ml of fresh acetone solution on the plant material. Extraction procedures were effective as > 95% of ^{14}C was recovered from plant residue based on oxidation results in pilot experiments. The two 0.75 ml of extract were combined per sample and solvent was evaporated with a heating block set for 30 C in a hood. Samples were then resuspended in 500 μl of acetone and spotted on 20 by 20-cm silica gel plates for thin layer chromatography analysis. Samples were developed to 16 cm in a glass chamber with 70 : 20 ethyl-acetate : hexane solution.

Plates were then air-dried and metabolites were identified with a radiochromatogram scanner (BioScan System 200 Imaging Scanner, Bioscan, 4590 MacArthur Boulevard NW, Washington, DC 20007) equipped with Laura Chromatography Data Collection and Analysis Software® (LabLogic Systems, Inc. 1040 E Brandon Blvd Brandon, FL 33511-5509) that measured radioactivity counts. The parent herbicide was detected at retention factor (R_f) of 0.7 by spotting stock solution suspended in 500 μl of acetone and scanning the developed plates. Metabolites with $R_f < 0.7$ were considered more polar than the parent herbicide and metabolites with $R_f > 0.7$ were considered less polar than the parent herbicide. Data were subjected to analysis of variance at the 0.05 probability level. Means were separated with Fisher's Protected LSD test at $\alpha = 0.05$.

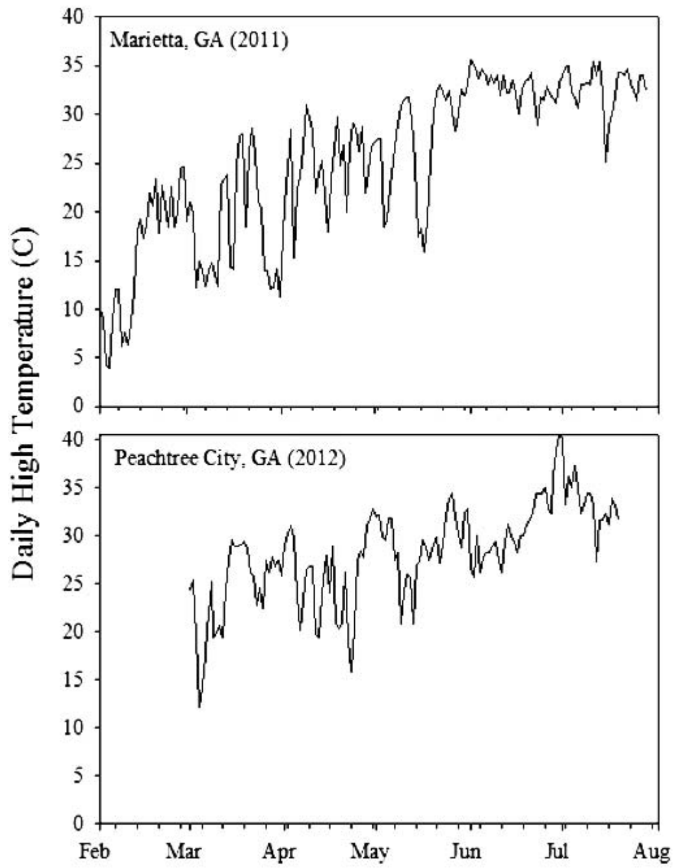

Figure 1. Daily high temperatures in Marietta, GA and Peachtree City, GA during field experiments in 2011 and 2012, respectively.

Results and Discussion

Field Experiments. Creeping bentgrass injury was minimal (< 8%) on all evaluation dates but methiozolin caused more

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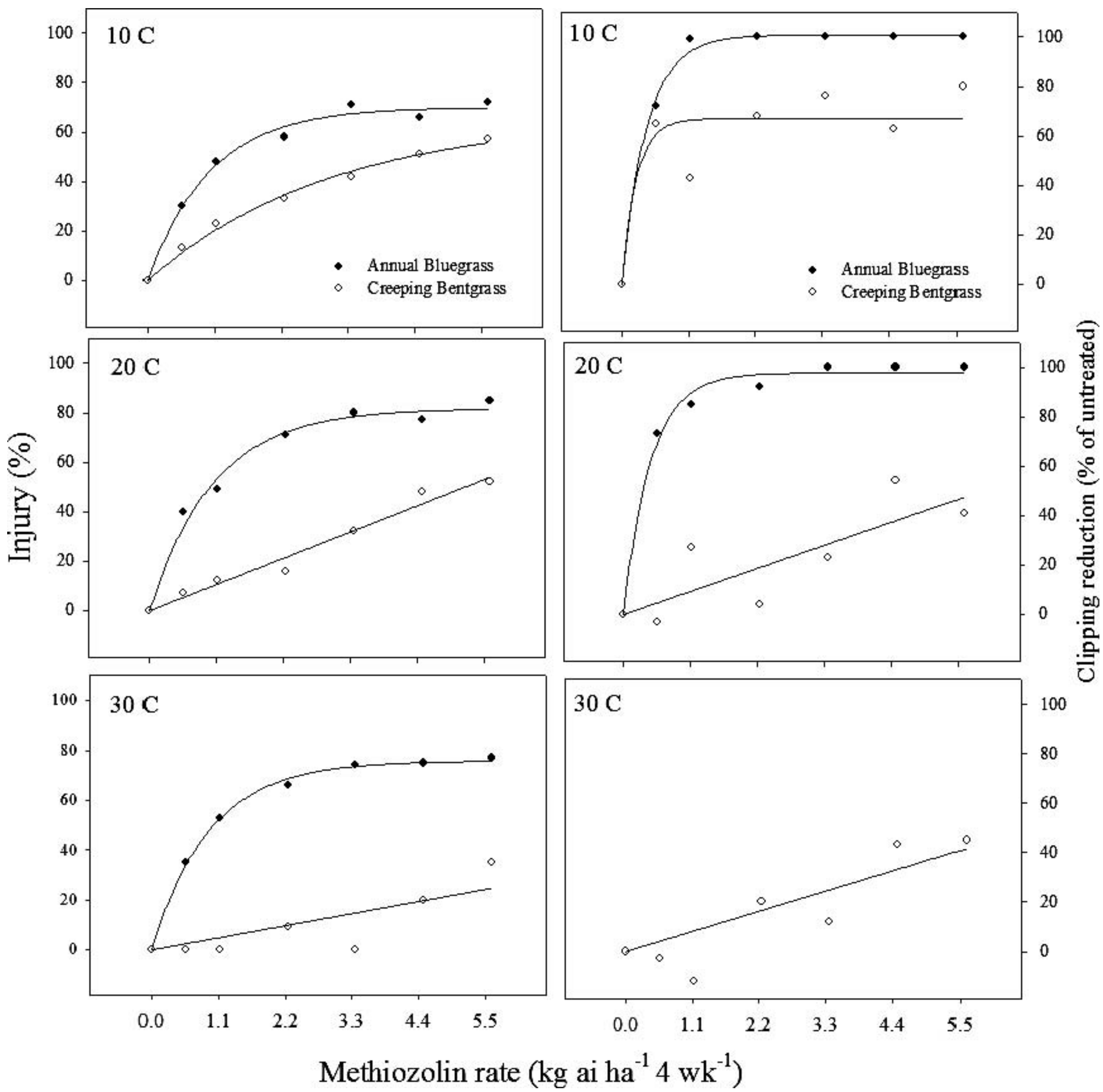

Figure 2. Percent injury and clipping reductions eight weeks after initial treatments of methiozolin applied sequentially in two combined growth chamber experiments, 2011 to 2012, Griffin, GA.

injury to creeping bentgrass from the high rate when applied in February/March compared to May (Table 1). Applications beginning in Feb/March at 1.68 kg ha^{-1} injured creeping bentgrass 6 and 7% at 2 and 4 WAIT, respectively, while this rate applied in May caused no injury. All other methiozolin rates caused $\leq$ 2% creeping bentgrass injury. Conversely, creeping bentgrass was more sensitive to amicarbazone at the May application timing compared to February/March timing. Creeping bentgrass was injured 6 and 5% at 2 and 4 WAIT, respectively, in May compared to $\leq$ 1% from amicarbazone treatments initiated in February/March.

The interaction of application timing and treatment was significant for annual bluegrass control at 4 and 8 WAIT (Table 1). Sequential methiozolin treatments at 0.84 followed by (fb) 0.84 kg ha^{-1} and 1.68 fb 1.68 kg ha^{-1} had similar annual bluegrass control at both application timings averaging 58% and 93% at 8 WAIT, respectively. Methiozolin applied sequentially at 0.42 fb 0.42 kg ha^{-1} controlled annual bluegrass 23% with the February/March timing but applications beginning in May provided 50% control at 8 WAIT. Applications of amicarbazone in February/March controlled annual bluegrass 17 and 23% by 4 and 8 WAIT, respectively, while treatments beginning in May provided 83% and 98% control at 4 and 8 WAIT, respectively.

Daily high temperatures from the initial February/March treatments ranged 11 to 31 C and 16 to 33 C until 8 WAIT in 2011 and 2012, respectively (Figure 1). After the May application, temperatures increased to 16 to 36 C and 25 to 41 C in 2011 and 2012, respectively. Annual bluegrass control from amicarbazone was substantially better under warmer temperatures and is consistent with previous research (McCullough et al. 2010). While temperature and application timing influenced amicarbazone efficacy, only methiozolin treatments at the lowest rate, 0.42 fb 0.42 kg ha^{-1}, had increased control at warmer temperatures. Results suggest that methiozolin could be applied in programs totaling 1.68 to 3.36 kg ha^{-1} under these temperature ranges without compromising efficacy for annual bluegrass control.

Although sequential methiozolin applications at 1.68 fb 1.68 kg ha^{-1} provided excellent (> 90%) annual bluegrass

Table 2. Predicted rates from regression equations to injure annual bluegrass 50% (I_{50}) and 'Penncross' creeping bentgrass 20% (I_{20}) and reduce clippings 50% from the untreated (CR_{50}) at eight weeks after initial treatments following two applications of methiozolin in two combined growth chamber experiments, 2011–2012, Griffin, GA.

Temperature	Injury: Annual bluegrass (I_{50})	Injury: Creeping bentgrass (I_{20})	Clipping reductions: Annual bluegrass (CR_{50})	Clipping reductions: Creeping bentgrass (CR_{50})
C	kg ai ha^{-1} [a]			
10	1.3	1.1	0.2	0.8
20	1.0	2.1	0.4	> 5.6
30	1.0	4.2	[b]	> 5.6
	Equations			
10	$r^2 = 0.85$, $y = 70.05[1 - {}^{(-0.97x)}]$, SE[c] = 3.1	$r^2 = 0.84$, $y = 68.26[1 - {}^{(-0.31x)}]$, SE = 1.9	$r^2 = 0.98$, $y = 100.05[1 - {}^{(-4.0x)}]$, SE = 12.6	$r^2 = 0.22$, $y = 70.51[1 - {}^{(-1.68x)}]$, SE = 3.8
20	$r^2 = 0.84$, $y = 81.49[1 - {}^{(-0.97x)}]$, SE = 4.2	$r^2 = 0.62$, $y = 0.095 + 9.66x$, SE = 3.5	$r^2 = 0.79$, $y = 99.2[1 - {}^{(-1.70x)}]$, SE = 2.6	$r^2 = 0.12$, $y = -7.15 + 8.03x$, SE = 9.4
30	$r^2 = 0.95$, $y = 75.62[1 - {}^{(-1.06x)}]$, SE = 1.5	$r^2 = 0.23$, $y = -6.16 + 6.11x$, SE = 7.7		$r^2 = 0.18$, $y = -10.24 + 9.36x$, SE = 10.7

[a] Two applications of methiozolin were applied with the second made after four weeks at 0, 0.56, 1.12, 2.24, 3.36, 4.48, or 5.60 kg ai ha^{-1}.
[b] Annual bluegrass had no clippings from any treatment, including the nontreated, at 30 C at 8 wk after initial treatments.
[c] SE = standard error of estimates.

control at both timings, an interaction with treatments and timing was detected at 8 WAIT for turf quality (Table 1). Applications of amicarbazone in February/March did not reduce turf quality from the untreated while treatments beginning in May reduced quality by 30% from the untreated. Similarly, sequential methiozolin applications at 1.68 fb 1.68 kg ha^{-1} in February/March did not reduce quality from the untreated but applications in May reduced quality 23%. However, sequential methiozolin applications at 0.42 fb 0.42 kg ha^{-1} and 0.84 fb 0.84 kg ha^{-1} did not reduce turf quality from the untreated at either timing.

Although later application timings of methiozolin caused minimal injury, reductions in turf quality probably resulted from creeping bentgrass growth inhibition and subsequent voids in plots during summer months following annual bluegrass control. Methiozolin applications in February/March were timed prior to the stressful period of summer heat that probably enabled creeping bentgrass to fill in voids after annual bluegrass control in spring (Huang and Gao 2000). Methiozolin applications totaling 0.84 and 1.68 kg ha^{-1} did not reduce turf quality at either timing but treatments were less effective for controlling annual bluegrass than a total of 3.36 kg ha^{-1}. Despite less control, suppression of annual bluegrass in creeping bentgrass golf greens could be desirable and warrants further research in programs at reduced rates. Overall, the best treatment from this experiment for annual bluegrass control, creeping bentgrass tolerance, and turf quality was methiozolin applied sequentially at 1.68 fb 1.68 kg ha^{-1} beginning in February/March when daily high temperatures ranged $\approx$ 15 to 25 C (Figure 1).

Growth Chamber Experiments. Species by temperature interactions were detected for injury and clipping reductions from the untreated at 8 WAIT, and thus, results are presented across temperatures by species. Creeping bentgrass had the most injury from methiozolin applications at 10 C compared to 20 C and 30 C (Figure 2). From regression analysis, creeping bentgrass I_{20} values (rate required to cause 20% injury) measured 1.1, 2.1, and 4.2 kg ha^{-1} at 10, 20, and 30 C, respectively (Table 2). Annual bluegrass had similar injury across the three temperatures as I_{50} values measured 1.3, 1.0, and 1.0 kg ha^{-1} at 10, 20, and 30 C, respectively.

Clipping reductions from the untreated were significantly influenced by temperature at 8 WAIT (Figure 2). Creeping bentgrass CR_{50} (rates required to reduce clippings 50% from untreated) measured 0.8, > 5.6, and > 5.6 kg ha^{-1} at 10, 20, and 30 C, respectively (Table 2). Annual bluegrass had significantly greater clipping reductions from the untreated compared to creeping bentgrass as CR_{50} measured 0.2 and 0.4 kg ha^{-1} at 10 and 20 C, respectively. Annual bluegrass had no clippings from any treatment, including the nontreated, at 30 C and therefore CR_{50} values could not be measured.

Creeping bentgrass responses to methiozolin in growth chamber experiments are consistent with field experiments as greater injury was noted with February/March applications compared to applications beginning in May. Results are likely attributed to increased sensitivity of creeping bentgrass to methiozolin at low temperatures and are similar to reports with bispyribac-sodium and sulfosulfuron (McCullough and Hart 2006, 2008). Annual bluegrass responses to methiozolin were comparable across the three temperatures tested and are

Table 3. Annual bluegrass and 'Penn A-4' creeping bentgrass total absorption and radioactivity distribution following foliar applications of ^{14}C-methiozolin in two combined laboratory experiments, 2012, Griffin, GA.

		Foliar absorption[a] (HAT[b])			^{14}C Distribution (24 HAT)			^{14}C Distribution (72 HAT)		
Temperature	Species	4	24	72	Treated leaf	Nontreated shoots	Roots	Treated leaf	Nontreated shoots	Roots
day/night (C)		% of applied			% of absorbed					
15/10	Annual bluegrass	7	13	15	90	6	4	92	4	4
	Creeping bentgrass	7	16	16	94	4	2	94	4	2
	$LSD_{0.05}$	NS	NS	NS	NS	NS	NS	NS	NS	NS
30/25	Annual bluegrass	12	20	35	95	3	2	96	3	1
	Creeping bentgrass	12	21	23	91	8	1	91	6	3
	$LSD_{0.05}$	NS	NS	11	NS	NS	NS	NS	NS	NS
Temperature		NS	*	*	NS	NS	*	NS	NS	NS
Species		NS	NS	*	NS	NS	NS	NS	NS	NS
Temperature × Species		NS	NS	*	NS	NS	NS	*	NS	*

* , NS = significant and not significant at the 0.05 probability level, respectively.

[a] Foliar absorption was measured by rinsing the treated leaf with 20% methanol solution followed by oxidizing the entire plant (treated leaf, nontreated shoots, and roots). Absorption was calculated by dividing the total amount of radioactivity recovered in the whole plant from radioactivity applied.

[b] HAT = hours after treatment.

similar to control in field experiments when ≥ 1.68 kg ha^{-1} total was applied in February/March or May. Similar responses of annual bluegrass to methiozolin at different temperatures suggest turf managers could have greater application timing flexibility compared to amicarbazone, bispyribac-sodium, and other herbicides that require specific temperature ranges to effectively control annual bluegrass (Anonymous 2012; Lycan and Hart 2006b; McCullough and Hart 2006; McCullough et al. 2010).

Absorption, Translocation, and Metabolism of ^{14}C-Methiozolin. Annual bluegrass and creeping bentgrass had similar foliar absorption of ^{14}C-methiozolin at 4 and 24 HAT but temperature effects were not significant until 24 HAT (Table 3). Both grasses averaged 6 to 12% more foliar absorption at 24 and 72 HAT when grown at 30/25 than 15/10 C. At 72 HAT, annual bluegrass absorbed more ^{14}C-methiozolin than creeping bentgrass when grown at 30/25 C, measuring 35% and 23% of applied, respectively, but species had similar absorption at 15/10 C, averaging 16%. Radioactivity distribution was similar across temperatures from foliar-applied methiozolin and meaningful interactions were not detected with species. Annual bluegrass had similar ^{14}C distribution from foliar applications at 24 and 72 HAT as > 90% of absorbed ^{14}C was recovered in the treated leaf (Table 3). Radioactivity distribution in roots and nontreated shoots was similar for annual bluegrass and creeping bentgrass at both temperatures at 24 and 72 HAT.

In root absorption experiments, species by temperature interactions were not detected for ^{14}C absorption, distribution, or recovery (Table 4). After 48 hr, grasses treated at 30/25 C absorbed more ^{14}C-methiozolin from roots than at 15/10 C, measuring 16 and 11% of the applied, respectively. Root absorption of ^{14}C-methiozolin was similar for the two grasses, averaging 14% of the applied, but annual bluegrass distributed more ^{14}C to shoots than creeping bentgrass, measuring 31% and 20% of the total per plant, respectively. Creeping bentgrass recovered about twofold more ^{14}C in roots than annual bluegrass, averaging 2973 and 1429 Bq g^{-1} dry weight, respectively. Moreover, annual bluegrass recovered 33% greater radioactivity in shoots than creeping bentgrass from root applications. Pooled over grasses, radioactivity distribution to shoots was about twofold higher at 30/25 C, averaging 34%, compared to plants at 15/10 C which had 18% distribution of the total absorbed ^{14}C to shoots.

Metabolites were not detected in annual bluegrass or creeping bentgrass on any evaluation date (data not shown). At both temperatures, metabolite recovery of annual bluegrass and creeping bentgrass measured the same R_f as the parent herbicide suggesting no metabolism had occurred at 1, 3, or 7

Table 4. Absorption, distribution, and recovery of radioactivity at 48 hours after root applications of ^{14}C-methiozolin for annual bluegrass and 'Penn A-4' creeping bentgrass in two combined growth chamber experiments, 2012, Griffin, GA.

	Root absorption	^{14}C distribution		^{14}C recovery	
	Whole plant	Roots	Shoots	Roots	Shoots
Species	% of applied[a]	% of absorbed		Bq g^{-1} dry wt	
Annual bluegrass	13	69	31	1429	335
Creeping bentgrass	14	80	20	2973	251
$LSD_{0.05}$	NS	4	5	467	67
Temperature (day/night)					
15/10 C	11	82	18	1749	168
30/25 C	16	66	34	2535	407
$LSD_{0.05}$	3	4	5	465	67
Species	NS	*	*	*	*
Temperature	*	*	*	*	*
Species × Temperature	NS	NS	NS	NS	NS

[a] Plants roots were submerged in 25 ml of half-strength Hoagland's solution spiked with ^{14}C-methiozolin at 15.2 kBq L^{-1} plus nonradiolabeled methiozolin within a 50-ml tube using cotton balls at the base of shoots. Total surface application rate was 1.12 kg ha^{-1}.

DAT. These findings of no metabolites with methiozolin are consistent with previous research (S. Koo, personal communication). However, the influence of temperature on methiozolin metabolism in annual bluegrass and creeping bentgrass has received limited investigation. Temperature effects on herbicide metabolism does not appear to be correlated with differential tolerance levels of annual bluegrass and creeping bentgrass to methiozolin. Results also suggest metabolism within a 7-d period is not related to selectivity of methiozolin for annual bluegrass control in creeping bentgrass turf.

Differential absorption and translocation between annual bluegrass and creeping bentgrass could be attributed to methiozolin selectivity and is similar to previous research with amicarbazone, bispyribac-sodium, and ethofumesate (Kohler and Branham 2002; Lycan And Hart 2006; Yu et al. 2013). However, differential metabolism of these herbicides in annual bluegrass and creeping bentgrass has been noted and does not appear consistent with efficacy of methiozolin (Kohler and Branham 2002; McCullough et al. 2009; Yu et al. unpublished data). Annual bluegrass distributed more root-absorbed methiozolin to shoots than creeping bentgrass but distribution from foliar applications was similar. Although root absorption was comparable between species, mature creeping bentgrass turf often has a deeper root system than annual bluegrass and greater relative absorption by a shallow-rooted plant could increase phytotoxic effects for control in polyculture with established turf species. Future research should evaluate application placement of methiozolin and differential responses of annual bluegrass and creeping bentgrass as influenced by plant maturity and rooting depth.

The influence of temperature was significant on methiozolin absorption and translocation as both species distributed more root-absorbed ^{14}C to shoots at warm versus cooler temperatures. Creeping bentgrass injury appears to be exacerbated by reductions in temperature but differences in absorption, translocation, and metabolism at various temperatures do not explain these responses and warrant further investigation. Annual bluegrass control was generally not affected by application timing or temperature at total rates ≥ 1.68 kg ha^{-1} suggesting methiozolin could provide greater application flexibility than other chemistries currently available for creeping bentgrass including amicarbazone, bispyribac-sodium, and ethofumesate. Overall, methiozolin appears effective for controlling annual bluegrass in creeping bentgrass golf greens at various temperatures and application timings, while selectivity appears attributed to differential absorption and translocation rather than metabolism.

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