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Absorption, Translocation, and Metabolism of Amicarbazone in Annual Bluegrass (*Poa annua*), Creeping Bentgrass (*Agrostis stolonifera*), and Tall Fescue (*Festuca arundinacea*)

Jialin Yu, Patrick E. McCullough, and William K. Vencill*

Amicarbazone controls annual bluegrass in cool-season turfgrasses but physiological effects that influence selectivity have received limited investigation. The objective of this research was to evaluate uptake, translocation, and metabolism of amicarbazone in these species. Annual bluegrass, creeping bentgrass, and tall fescue required < 3, 56, and 35 h to reach 50% foliar absorption, respectively. At 72 h after treatment (HAT), annual bluegrass and creeping bentgrass translocated 73 and 70% of root-absorbed ^{14}C to shoots, respectively, while tall fescue only distributed 55%. Annual bluegrass recovered ≈ 50% more root-absorbed ^{14}C in shoots than creeping bentgrass and tall fescue. Creeping bentgrass and tall fescue metabolism of amicarbazone was ≈ 2-fold greater than annual bluegrass from 1 to 7 d after treatment (DAT). Results suggest greater absorption, more distribution, and less metabolism of amicarbazone in annual bluegrass, compared to creeping bentgrass and tall fescue, could be attributed to selectivity of POST applications.

Nomenclature: Amicarbazone; annual bluegrass, *Poa annua* L.; creeping bentgrass, *Agrostis stolonifera* L.; tall fescue, *Festuca arundinacea* Schreb.

Key words: Efficacy, selectivity, turfgrass.

Annual bluegrass is a problematic weed that reduces aesthetics and functionality of cool-season turfgrasses (Beard 1970; Sprague and Burton 1937). Compared to most turf species, annual bluegrass has a lighter green color, coarser leaf texture, and produces unsightly seedheads that reduce turfgrass quality (Lush 1989). Additionally, annual bluegrass has poor disease, heat, and drought tolerances that may increase requirements for water, fungicides, and intensive management in successful turfgrass culture (Beard 1970; Lush 1989).

PRE herbicides often provide erratic annual bluegrass control and POST herbicides have limitations on selectivity in cool-season turfgrasses (Callahan and McDonald 1992; Juska and Hanson 1967). Bispyribac-sodium is an acetolactate synthase inhibitor with potential for controlling annual bluegrass in cool-season grasses but applications are limited to spring and early summer to maximize efficacy for turfgrass tolerance and control (Lycan and Hart 2006). Ethofumesate, mesotrione, and sulfosulfuron may control immature annual bluegrass, but inconsistent efficacy and turf injury limit potential for use of these herbicides in cool-season grasses (Jones and Christians 2007; Lycan and Hart 2004; Lycan et al. 2005; Meyer and Branham 2006).

Amicarbazone is a triazolinone herbicide used for weed control in corn (*Zea mays* L.) and sugarcane (*Saccharum officinarum* L.) (Dayan et al. 2009; Philbrook et al. 1999; Seeruttun et al. 2008). Amicarbazone inhibits photosynthesis and phytotoxic symptoms on susceptible species are similar to triazines including chlorosis, stunted growth, and necrosis of leaves (Dayan et al. 2009; Senseman 2007). Recently, researchers noted amicarbazone has potential to selectively control annual bluegrass in turfgrasses (McCullough et al. 2010; Perry et al. 2011). Injury of cool-season turfgrasses from amicarbazone may increase with elevated temperatures from 10 to 30 C but spring applications can be effective for selectively controlling annual bluegrass (McCullough et al. 2010). Amicarbazone may be safely applied at 0.2 kg ai ha^{-1} to tall fescue but end-users are limited to sequential applications of 0.05 kg ha^{-1} on creeping bentgrass because of turfgrass injury (Anonymous 2012).

Although amicarbazone has potential for POST annual bluegrass control, limited research has been conducted on parameters attributed to selectivity of the herbicide for use in cool-season turfgrass. Thus, comprehensive investigations are required to evaluate amicarbazone physiology in turfgrass to help maximize efficacy for annual bluegrass control. The objective of this research was to evaluate absorption, translocation, and metabolism of amicarbazone in annual bluegrass, creeping bentgrass, and tall fescue.

Materials and Methods

Foliar Absorption and Translocation. Two separate experiments were conducted in Athens, GA from October to December 2011. Single tillers of annual bluegrass, 'Penncross' creeping bentgrass, and 'Talladega' tall fescue were transplanted from field samples in Griffin, GA in pots (3.8 cm diam and 20 cm deep) filled with sand : peat moss (80 : 20 v/v). Annual bluegrass was an indigenous biotype taken from fields without a history of resistance to photosynthesis inhibiting herbicides. After resuming active growth in a greenhouse, grasses were placed in a growth chamber (Environmental Growth Chambers®, P.O. Box 407, Chagrin Falls, OH 44022) set for 25/20 C day/night with 470 µmol m^{-2} s^{-1}, and photoperiod of 12 h. Plants were watered to prevent wilting and trimmed as needed with sheers to maintain 5 cm heights. Grasses were allowed to produce three to five new tillers prior to treatments and plants were selected based on size and population uniformity.

A broadcast application of nonlabeled amicarbazone (70WDG, Arysta LifeScience, Suite 150 Cary, NC 27513) was made at 0.2 kg ai ha^{-1} to grasses in a spray chamber at 374 L ha^{-1}. Immediately after the broadcast application, a 2-µl droplet of spotting solution containing a total of 150 Bq of ^{14}C-amicarbazone (uniformly ring-labeled [specific activity, 39.6 mCi $mmol^{-1}$, 99.1% radiochemical purity]) was applied to first fully developed leaf with a 5-µl microsyringe

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* First, second, and third authors: Graduate Student, Assistant Professor, and Professor, respectively, Department of Crop and Soil Sciences, University of Georgia, Griffin, GA 30223-1797; Corresponding author's Email: pmccull@uga.edu

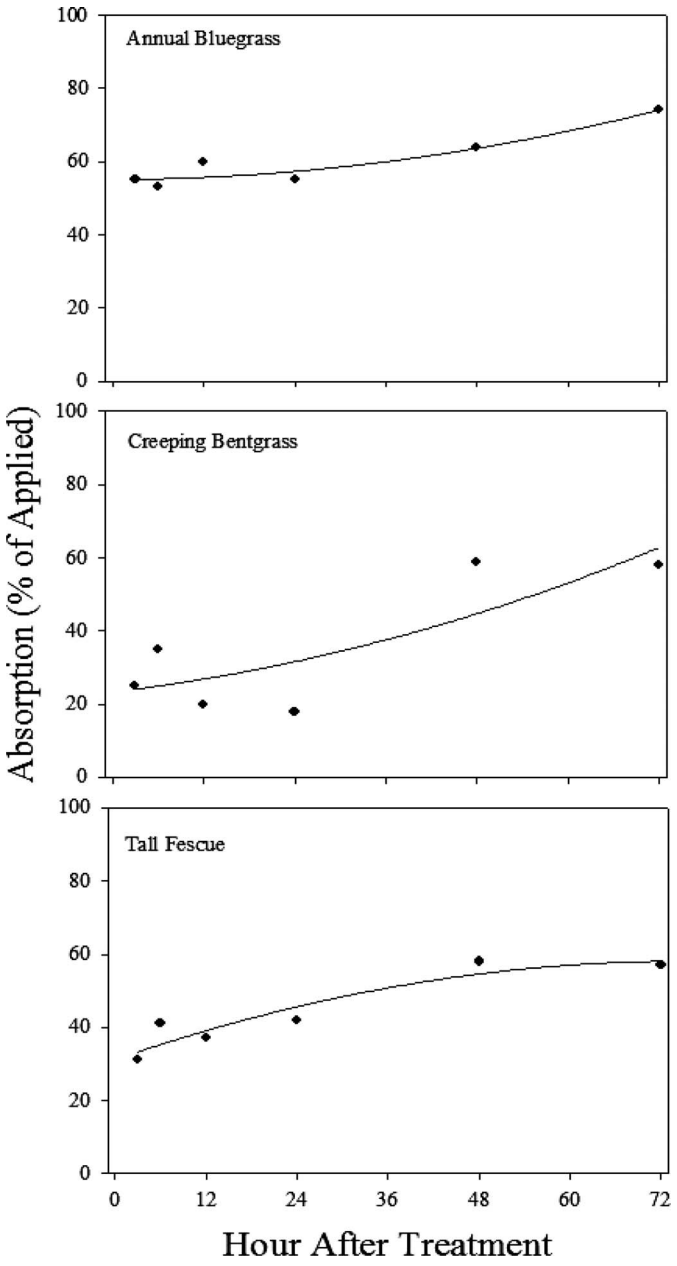

Figure 1. Absorption of foliar applied ^{14}C-amicarbazone in two combined growth chamber experiments, 2011, Athens, GA.

(Hamilton Co., Reno, NV 89502). Formulated amicarbazone was added to the spotting solution to simulate a spray droplet from broadcast treatments with 0.2 kg ai ha^{-1} at 374 L ha^{-1}. Nonionic surfactant (NIS) (Activator 90®, Loveland Industries, Greeley, CO 80631) was added to the spotting solution at 0.125% v/v to facilitate deposition of the droplets on leaves.

Grasses (shoots + roots) were harvested at 3, 6, 12, 24, 48, and 72 h after treatment (HAT). Plants harvested at 24, 48, and 72 HAT were sectioned into treated leaf, nontreated shoots, and roots. Unabsorbed ^{14}C-amicarbazone was removed by swirling the treated leaf in a 20 ml scintillation vial (1654 High Hill Road, Swedesboro, NJ 08085) containing 2 ml of 10% methanol solution followed by rinsing with an additional 2 ml of methanol solution. The plant segments were oven dried at 65 C for 48 h and combusted in a biological oxidizer (Harvey Biological Oxidizer®, OX-500, R. J. Harvey Instrument Corp., 11 Jane Stree, Tappan, NY 10983). Radioactivity in the oxidized samples was analyzed using a Liquid Scintillation Counter (LSC) (Beckman LS 6500®, Beckman Coulter Inc., Fall River, MA 02720). Percent foliar absorption was determined by dividing the total ^{14}C recovered in plants by the amount of ^{14}C applied.

Experimental design was completely randomized with four replications and the experiment was repeated. Data was subjected to analysis of variance (ANOVA) with SAS (100 SAS Campus Dr., Cary, NC 27513) and significance of main effects was determined at the 0.05 probability level. Means were separated using Fisher's Protected LSD test at $\alpha = 0.05$. Absorption data was analyzed with regression analysis, $Y = B(0) + B(1)X + B(2)X^2$ in Sigma Plot 11.2 (Systat Software, Inc., 225 W. Washington St., Chicago, IL 60606). Time required to achieve 50% foliar absorption (t_{50}) was determined for each species. Experiment-by-treatment interactions were not detected, and thus, results were pooled over experiments.

Root-Applied Absorption and Translocation. Two experiments were conducted in Athens, GA from October to December 2011 with aforementioned plant material. Once grasses had produced three to five new tillers, soil was washed from roots and grasses were placed in a hydroponic tank containing 3 L of half-strength Hoagland solution (Hoagland and Arnon 1950). Roots were submerged in solution by placing grasses through holes in a styrofoam board (Williams Foam, 12961 San Fernando Road, Sylmar, CA 91342) that completely covered the 5-L tank. Sides of the tank were covered with aluminum foil to shield roots from light and an aquarium pump (Shkerry Auqa®, Shanghai Uni-Aqua Co., Ltd, Chang Shou Road, Shanghai 200042, China) was used to provide plant roots with oxygen. Plants were acclimated in the solution for 7 d.

After acclimation, plants were placed into 50 ml plastic tubes filled with 25 ml half strength Hoagland solution spiked with 26.7 kBq L^{-1} of ^{14}C-amicarbazone. This concentration was selected to ensure radioactivity levels were sufficient to quantify uptake and translocation in samples. Nonlabeled amicarbazone was added to the solution to bring the surface application rate to 0.2 kg ai ha^{-1}. Tubes were covered with aluminum foil to prevent root exposure to light and grasses were suspended in tubes using cotton balls at the base of shoots.

Grasses were harvested at 72 HAT and sectioned to roots and shoots. Roots were rinsed thoroughly with 10% methanol solution and then blotted on a towel. Samples were then oven dried at 60 C for 48 h, weighed, and oxidized with aforementioned methods. Radioactivity was quantified with LSC and presented as radioactivity per gram of dry weight. Radioactivity per dry weight for total plant was converted with the following equation:

$$\text{Total } ^{14}\text{C recovery} = (\text{radioactivity in shoots} + \text{radioactivity in roots})/(\text{weight of shoots} + \text{weight of roots})$$

Experimental design was completely randomized with six replications. Significance of main effects was determined using ANOVA in SAS (SAS Institute Inc., Cary NC 27513). Means were separated using Fisher's Protected LSD test at $\alpha = 0.05$.

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Table 1. Predicted amount of time required to achieve 50% foliar absorption for three grasses treated with ^{14}C-amicarbazone in two combined growth chamber experiments, 2011, Athens, GA.

Time to Reach 50% Foliar Absorption		
Annual Bluegrass	Creeping Bentgrass	Tall Fescue
HAT[a]		
< 3	56	35
Equations		
$r^2 = 0.21$ $y = 55.86 - 0.35x + 0.009x^2$	$r^2 = 0.49$, $y = 22.9 + 0.26x + 0.004x^2$	$r^2 = 0.38$ $y = 31.2 + 0.68x - 0.004x^2$

[a] HAT = H after treatment.

Experiment-by-treatment interactions were not detected, and thus, experiments were combined.

Metabolism Experiments. Two experiments were conducted in Griffin, GA from July to August 2012. Annual bluegrass, creeping bentgrass, and tall fescue plant material was prepared and grown with aforementioned methods and placed in a growth chamber set for 25/20 (day/night) with a 12 h photoperiod of 400 µmol m^{-2} s^{-1}. After acclimation for one wk, plants were treated with a broadcast application of nonlabeled amicarbazone at 0.2 kg ha^{-1} with a CO_2-pressured sprayer (Tee Jet®, Spraying Systems Co., Wheaton, IL 60189-7900). Immediately after broadcast applications, a 2 µl droplet containing approximately 1.7 kBq of ^{14}C-amicarbazone with 0.125% v/v NIS was applied to the adaxial surface of a single leaf of each grass with a 5-µl microsyringe. Treated plants were harvested at 1, 3, and 7 d after treatments (DAT). Unabsorbed ^{14}C was washed off the treated leaf using methods previously described. Treated leaves were stored at −10 C in a freezer immediately after harvesting.

Individual leaves were placed in 1.5 ml microcentrifuge tubes (Eppendorf , Fisher Scientific, Fair Lawn, NJ 07410) and ground with liquid nitrogen. Tubes with ground leaf tissue were filled with 0.5 ml of hexane ; isopropanol : acetic acid (70 : 29 : 1). Tubes were placed in water sonication for 45 min. Samples were then centrifuged for 5 min and solution was transferred to separate tubes. This procedure was conducted three times with fresh solvent added to ground tissue and extraction solutions were combined in a 1.5 ml tube. Residues in tubes were air dried and oxidized to test extraction efficiency.

Extraction solvent was then placed in glass vials and heated to 55 C under a fume hood to evaporate the solvent. Samples were allowed to cool and then resuspended in 1 ml of dichloromethane. Subsequently, a 500 µl aliquot was spotted on 20 cm length silica gel thin layer chromatography (TLC) plates and developed to 16 cm with ethyl acetate : dichloromethane : acetic acid (20 : 70 : 2 v/v/v). TLC plates were then air-dried, and radioactive trace peaks were determined by scanning plates with a radiochromatogram scanner (Bioscan 2000, Bioscan, Inc., 4590 MacArthur Blvd., Washington, DC 20007). Stock radiolabeled herbicide solution was dissolved in 500 µl of dichloromethane, developed on TLC plates, and the parent herbicide was identified at $R_f = 0.55$. Peaks in radioactivity on TLC plates were identified using Laura Chromatography Data Collection and Analysis Software® (LabLogic Systems, Inc. 1040 E Brandon Blvd Brandon, FL 33511-5509) connected to the scanner. Metabolites were classified in three groups including parent herbicide, total percentage of metabolites more polar than the parent herbicide, and total percentage of metabolites less polar than the parent herbicide. Data were subjected to analysis of variance (ANOVA) and means were separated using Fisher's Protected LSD test at $\alpha = 0.05$. Experiment by treatment interactions were not detected, and thus experiments were combined.

Results and Discussion

Foliar Absorption and Translocation. Foliar absorption generally increased from 3 to 72 HAT and ranged 56 to 74%, 19 to 59%, and 25 to 59% for annual bluegrass, creeping bentgrass, and tall fescue, respectively (Figure 1). From regression analysis, annual bluegrass, creeping bentgrass, and tall fescue required < 3, 56, and 35 h to absorb 50% of the applied herbicide, respectively (Table 1). Results suggest annual bluegrass has faster and more foliar absorption of amicarbazone than creeping bentgrass and tall fescue. Foliar uptake of herbicides is related to leaf properties such as cuticle thickness, epicuticular waxes, leaf maturity, and stomata numbers (Chachalis et al. 2001; Hess 1985; Kalnay and Glenn 2000; Sanyal et al. 2006; Wanamarta and Penner 1989). Greater foliar absorption of amicarbazone in annual bluegrass, compared to creeping bentgrass and tall fescue, could be attributed to these properties and may influence differential tolerance levels among species.

At 24 and 48 HAT, annual bluegrass distributed more foliar absorbed ^{14}C to roots (37 and 26%) than creeping

Table 2. Translocation of foliar-applied ^{14}C-amicarbazone in annual bluegrass, 'Penncross' creeping bentgrass, and 'Talladega' tall fescue in two combined growth chamber experiments, 2011, Athens, GA. Different letters indicate significant differences according to Fisher's Protected LSD test at $\alpha = 0.05$ by column.

	Distribution of Recovered ^{14}C in Plant Tissue (HAT)[a]								
	24			48			72		
Species	Treated Leaf	Nontreated Shoots	Roots	Treated Leaf	Nontreated Shoots	Roots	Treated Leaf	Nontreated Shoots	Roots
	% of Absorbed ^{14}C								
Annual Bluegrass	47 b	16 b	37 a	51 b	23 a	26 a	55 b	23 b	22 a
Creeping Bentgrass	48 b	40 a	12 c	59 a	21 a	20 b	50 b	30 a	20 a
Tall Fescue	63 a	16 b	21 b	62 a	23 a	15 c	75 a	15 c	10 b

[a] HAT = H after treatment.

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Table 3. Distribution and specific radioactivity of plant parts at 72 h after root applications of ^{14}C-amicarbazone to three grasses in two combined growth chamber experiments, 2011, Athens, GA. Different letters indicate significant differences according to Fisher's Protected LSD test at $\alpha = 0.05$.

	^{14}C Distribution		Specific Radioactivity		
	Roots	Shoots	Roots	Shoots	Total[a]
	% of Absorbed		Bq/g dry wt		
Annual Bluegrass	27 b	73 a	250 a	290 a	261 a
Creeping Bentgrass	30 b	70 a	230 a	173 b	197 b
Tall Fescue	45 a	55 b	201 a	198 b	192 b

[a] Total plant values = (radioactivity in roots + radioactivity in shoots) /(weight of roots + weight of shoots) for each individual plant.

bentgrass (12 and 20%) and tall fescue (21 and 15%, respectively) (Table 2). Conversely, tall fescue retained more foliar-absorbed ^{14}C in the treated leaf than annual bluegrass and creeping bentgrass at all harvest timings. From 24 to 72 HAT, the treated leaf of tall fescue retained 62 to 75% of the total ^{14}C recovered, whereas annual bluegrass and creeping bentgrass only had 47 to 55% and 48 to 59% in treated leaves, respectively. By 72 HAT, 30% of foliar-absorbed ^{14}C was in nontreated shoots of creeping bentgrass, but only 15% was in nontreated shoots of tall fescue. Generally, ^{14}C concentrations increased in the treated leaf of all grasses at 48 and 72 HAT compared to 24 HAT, which could be from greater foliar penetration at later timings. Delayed herbicide penetration reduces the rate of herbicide translocation from the treated leaf compared to species with faster foliar uptake (Kalnay and Glenn 2000). Greater herbicide translocation to annual bluegrass roots could increase herbicidal effects in meristematic tissues compared to creeping bentgrass and tall fescue.

Root Absorption and Translocation. At 72 HAT of root applications, annual bluegrass and creeping bentgrass distributed 73 and 70% of the total ^{14}C recovered to shoots, respectively, while tall fescue recovered only 55% in shoots (Table 3). Conversely, tall fescue retained 45% of total ^{14}C recovered in roots and was greater than annual bluegrass and creeping bentgrass (27 and 30% recoveries, respectively). The amount of ^{14}C recovered in roots (Bq g^{-1}) was similar in the three species but annual bluegrass had ≈50% more radioactivity recovered in shoots than creeping bentgrass and tall fescue. Similarly, total ^{14}C recovery of annual bluegrass was significantly greater than creeping bentgrass and tall fescue measuring 261, 197, and 192 Bq g^{-1}, respectively.

Results suggest annual bluegrass may distribute more root-absorbed amicarbazone to shoots than creeping bentgrass and tall fescue. Differences in translocation of amicarbazone from roots to shoot meristematic regions may affect the efficacy of root-applied amicarbazone and are consistent with application placement experiments. Perry et al. (2011) noted soil-applied amicarbazone controlled annual bluegrass 57 and 100%, respectively, at 1 and 3 wk after treatment. In contrast, the researchers noted foliar applications had slower activity and controlled annual bluegrass 34 and 93%, respectively, at 1 and 3 wk after treatment. Results suggest annual bluegrass may respond quicker to amicarbazone when absorbed by roots compared to shoots.

Metabolism. Extraction of ^{14}C was efficient as < 10% of absorbed ^{14}C-amicarbazone was recovered in plant residues (data not shown). The R_f value of amicarbazone was 0.55 and two major metabolites were detected at R_f values of 0.4 and 0.9. Another polar metabolite was detected at 0.15 but was generally < 10% of total metabolites recovered. Parent herbicide recovery declined from 1 to 7 DAT, suggesting metabolism increased with time in all species (Table 4). From 1 to 7 DAT, metabolism of ^{14}C-amicarbazone differed among species but annual bluegrass recovered approximately twice as much parent herbicide than creeping bentgrass and tall fescue.

At 1 DAT, annual bluegrass, creeping bentgrass, and tall fescue had 49, 17, and 25% parent herbicide recovered, respectively, and levels were comparable at 3 DAT (Table 4). By 7 DAT, parent herbicide recovery declined to 27% in annual bluegrass but was greater than creeping bentgrass and tall fescue recoveries of 11 and 14% of total metabolites, respectively.

Polar metabolite recovery was inconsistent across species at 1 and 3 DAT but creeping bentgrass and tall fescue recovery was ≈ 2-fold greater than annual bluegrass at 7 DAT. Nonpolar metabolite recovery was also significantly different in creeping bentgrass and tall fescue compared to annual bluegrass. At 1 DAT, nonpolar metabolite recovery in creeping bentgrass and tall fescue was ≈ 2-fold greater than annual bluegrass (Table 4). After 3 and 7 DAT, polar metabolites measured 38 and 51% of the total recovered in creeping bentgrass and tall fescue, respectively, and were significantly greater than 26% recovery of annual bluegrass. After 3 and 7 DAT, nonpolar metabolite recovery in annual bluegrass was generally similar to creeping bentgrass but tall fescue recovered significantly more metabolites than these species.

Differential species metabolism has been reported as the mechanism of selectivity in several herbicides used in grass crops. McCullough et al. (2009) noted differential metabolism levels of bispyribac-sodium could be attributed to selectivity for annual bluegrass control in creeping bentgrass

Table 4. Metabolism of foliar-applied ^{14}C-amicarbazone in annual bluegrass, creeping bentgrass, and tall fescue at 1, 3, and 7 d after treatment in two combined experiments, 2012, Griffin, GA. Different letters indicate significant differences according to Fisher's Protected LSD test at $\alpha = 0.05$ by column.

	Metabolites								
	1 DAT[a]			3 DAT			7 DAT		
Species	Polar[b]	Parent	Nonpolar	Polar	Parent	Nonpolar	Polar	Parent	Nonpolar
	% of total								
Annual Bluegrass	26b	49a	25b	35a	44a	21b	31b	27a	42a
Creeping Bentgrass	38a	17b	45a	47a	23b	30ab	51a	11b	38a
Tall Fescue	32ab	25b	43a	41a	24b	35ab	60a	14b	26b

[a] DAT = d after treatment.

[b] Metabolites: Polar, percentage sum of metabolites more polar than amicarbazone; Nonpolar, percentage sum of metabolites less polar than amicarbazone; and Parent, percentage sum of chemicals have similar polarity to amicarbazone.

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and perennial ryegrass (*Lolium perenne* L.). Olson et al. (2000) found metabolic rate of sulfosulfuron (MON 37500) in wheat (*Triticum aestivum* L.) was greater than downy brome (*Bromus tectorum* L.) and wild oat (*Avena fatua* L.). In other experiments, tolerance of feral rye (*Secale cereale* L.) to imazamox was accredited to greater metabolism than a sensitive species, jointed goatgrass (*Aegilops cylindrica* Host.) (Pester et al. 2000).

In conclusion, amicarbazone selectivity for annual bluegrass control in cool-season turfgrasses could be attributed to differential levels of absorption, translocation, and metabolism. Annual bluegrass appears to have more absorption and translocation of foliar and root-applied amicarbazone than creeping bentgrass and tall fescue. Amicarbazone is readily taken up by roots and translocated to shoots of annual bluegrass in greater concentrations than creeping bentgrass and tall fescue. Additionally, annual bluegrass appears to have less metabolism of amicarbazone than creeping bentgrass and tall fescue which could influence differential tolerance levels of herbicidal effects on these species.

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